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Invited Keynote Lecture

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IL-1 and inflammatory neurodegeneration

N. Rothwell
University of Manchester, Manchester, UK

Inflammation occurs rapidly in response to acute brain insults such as stroke, haemorrhage or trauma, and can be sustained for long periods of time for example in Alzheimer's, Parkinson's and multiple sclerosis. Experimental evidence indicates that inflammation plays a major role in neurodegeneration in these conditions and that the cytokine interleukin-1 (IL-1) is a pivotal mediator. IL-1 is expressed rapidly after neuronal injury, predominantly by microglia and elevated levels of endogenous or exogenous IL-1 markedly exacerbates injury. The naturally occurring IL-1 receptor antagonist (IL-1RA) markedly inhibits ischaemic, excitotoxic and traumatic brain injury in rodents, and has shown promise in a Phase II clinical trial in stroke patients.

The mechanisms of IL-1 expression, release and action in neurodegeneration are not fully elucidated and appear multiple. Systemic IL-1 markedly enhances ischaemic brain injury via release of neutrophils into circulation, neutrophil adhesion to injured cerebrovasculature and CNS invasion. Activation of matrix metalloproteinase 9 (MMP-9) occurs rapidly in neutrophils which have entered the CNS leading to cleavage of extracellular matrix and neuronal injury. IL-1 expressed within the CNS (primarily by microglia) acts on astrocytes to release neurotoxins including MMP-9, thus killing neurones indirectly via release of plasminogen. IL-1 can also influence neurones directly via non-classical signalling pathways.

Inflammation may also predispose to or trigger cerebrovascular events, though its role in repair and regeneration after brain injury is largely unknown.

Parallel Session 1: NK Cells and the Control of Infection

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Vaccines and innate immunity: lessons from cytomegalovirus immunoevasion of NKG2D

S. Jonjic
Histology and Embryology, Medical Faculty University of Rijeka, Rijeka, Croatia

NKG2D is a potent activating receptor expressed by cells of innate and adaptive immunity that recognizes cell surface molecules structurally related to MHC-I proteins induced by infection or other type of cellular stress. Engagement of NKG2D leads to cytotoxicity and cytokine secretion by NK cells, or to costimulation of $CD8^+$ T cells. Both human cytomegalovirus (CMV) and mouse CMV deployed evasive mechanisms to prevent expression of NKG2D ligands. The importance of viral regulation of NKG2D signaling pathway is illustrated by the fact that several herpesviruses, including human CMV, also use microRNA to regulate the expression of NKG2D ligands. So far we have characterized four mouse CMV proteins involved in the down-modulation of NKG2D ligands in infected cells. Deletion of any of these viral immunoevasive genes involved in regulation of NKG2D ligands resulted in virus attenuation *in vivo*. Based on the attenuation of viruses lacking NKG2D immunoevasins we proposed that the insertion of genes encoding NKG2D ligands in place of genes encoding their viral inhibitors, could be an appropriate approach for immunological attenuation of live vaccine. We have recently shown that despite the strong NK cell-mediated attenuation, mouse CMV engineered to express NKG2D ligand RAE-1gamma elicits a strong and long-lasting antiviral CD8 response, providing protection against lethal virus challenge (Slavuljica *et al.*, JCI 2010). In this talk I will overview the current knowledge on CMV downregulation of NKG2D signaling and our recent results using CMV expressing NKG2D ligand as a live attenuated vaccine and vaccine vector.

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Natural killer cells and hepatitis C virus infection

S. Khakoo
University of Southampton, Southampton, UK

Hepatitis C virus (HCV) is a common chronic viral infection that leads to cirrhosis, liver failure and hepatocelullar carcinoma. During the acute phase of HCV infection NK cells are activated, and in chronic HCV infection a number of phenotypic and functional abnormalities have been noted. Changes in acute and chronic HCV infection have been described both for cell surface receptor expression and also the activation status of NK cells.

Immunogenetic analyses have shown that specific combinations of killer-cell immunoglobulin-like receptors (KIR) and their MHC class I ligands are protective against chronic infection and also associated with a beneficial response to interferon-alpha. In order to investigate how the inhibitory KIR may affect the response to HCV infection, we have been studying the peptide specificity of KIR-positive NK cells. We have shown that KIR-positive NK cells are unexpectedly sensitive to changes in the peptide content of MHC class I. Specifically MHC class I:peptide complexes that engage inhibitory KIR weakly, can antagonise the effect of peptides that engage KIR strongly.

We have also shown that inhibitory peptides induce tight clustering of inhibitory KIR at the interface between NK cells and target cells, whereas antagonistic peptides induce a much more diffuse pattern of recruitment. This tight clustering requires SHP-1 phosphatase. The tight clustering observed with inhibitory peptides is disrupted by antagonistic peptides and hence inhibitory signalling prevented. Our data suggest that peptide antagonism of NK cells provides a rationale for the protective effect of inhibitory KIR in viral infections.

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Natural killer cells as effectors of vaccine-induced immunity?

E. Riley
Immunology and Infection, London School of Hygiene and Tropical Medicine, London, UK

Acquired immunity to infection is conferred by clonally-expanded populations of lymphocytes expressing surface antigen receptors with high affinity for the particular pathogen. Antigen-specific $CD4^+$ 'helper' T cells augment B cell, $CD8^+$ T cell and macrophage-mediated effector functions. We have recently identified an additional, little-appreciated but potentially very important, function for $CD4^+$ T helper cells, namely their ability to activate NK cells in an IL-2-dependent manner.

Activation of NK cells by various pathogens – including malaria-infected red blood cells, rabies virus, and Bacillus Calmette-Guérin (BCG) – is absolutely dependent upon IL-2 emanating from antigen-specific, MHC Class II-restricted $CD4^+$ T cells. Moreover, these NK cell responses are induced or augmented by vaccination, are re-activated within 6 h of re-exposure to the pathogen, represent the majority IFN-g-secreting and degranulating cells in the first 18 h after rechallenge, and contribute significantly to the effector response for at least 7 days. Thus, we hypothesise that an important function of effector/memory $CD4^+$ T cells may be to secrete IL-2, activating NK cells to secrete cytokines and become cytolytic. The size and speed of this 'recall' NK cell response suggests that it may represent a critical component of the immediate effector response to reinfection or post-vaccination.

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Supramolecular dynamics of natural killer cell immune synapses revealed by super-resolution microscopy

D. Davis
Division of Cell and Molecular Biology, Imperial College London, London, UK

Immune cell interactions are often accompanied by the segregation of proteins into micrometer- and submicrometer-scale domains at an immune synapse. T cell receptor signalling, for example, is initiated within structures termed microclusters within immune synapses. In Natural Killer (NK) cells, phosphorylation of inhibitory Killer Ig-like receptors (KIR) is similarly restricted to microclusters indicating that inhibitory signalling is also spatially restricted. And so the emerging new paradigm is that interactions between immune cell receptors, kinases and adaptors are at least in part controlled by the dynamics of supramolecular assemblies. Here we present data using new high-resolution imaging techniques to probe the dynamic synaptic organisation of NK cell receptors and filamentous (F)-actin. A combination of optical tweezers and live cell confocal microscopy reveals how microclusters of KIR and NKG2D are organised. Using 3D-structured illumination microscopy to gain super-resolution of ~100 nm, cortical actin was detected at the centre of the NK cell synapse, irrespective of whether activating or inhibitory signals dominate. But strikingly, the periodicity of the cortical actin mesh increased in specific domains at the centre of synapses in which activating signalling dominates. Two-colour super-resolution imaging revealed that lytic granules docked precisely in these domains which were also proximal to where the microtubule-organising centre (MTOC) polarised. Together, these data demonstrate that remodelling of cortical actin occurs at immune synapses during secretion. This is likely to occur for other types of cell secretion and emphasises the importance of emerging super-resolution imaging technology for revealing new biology.

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Immunogenetic variation characterizing exceptional control of HIV

M. Carrington

Cancer and Inflammation Program, Laboratory of Experimental Immunology, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD, USA

Some individuals infected with HIV-1 are able to control the virus without medication. These 'elite controllers' provide a unique opportunity to elucidate immune mechanisms that control HIV infection, which could have implications for the development of effective vaccines. We have been examining host genetic polymorphisms in a cohort of these individuals in order to define immune factors that might contribute to viral control. To date, genetic variability at the HLA class I loci has shown the greatest influence on outcome to HIV infection. In addition to the a-1 and a-2 domains of HLA class I, we have now begun to explore polymorphisms outside this region, as well as level of surface expression for their association with HIV diseases. Our focus has been on HLA-C, which has been largely ignored in HIV disease. We recently identified a polymorphic miRNA target site in the 3′UTR of HLA-C. Common SNPs in the miRNA-binding site regulate binding of miRNA and subsequent post-transcriptional processing, resulting in high or low HLA-C expression. A significant protective effect against HIV was observed amongst HLA-C alleles that escape miRNA mediated downregulation. Evolutionary studies indicate that the miRNA binding site polymorphism arose through a gene conversion event, which occurred 4–5 million years ago, and has been under selection pressure since that time, perhaps due to other infectious diseases. These findings add another dimension to the extreme polymorphism in HLA Class I loci that may account for variability in immune responses across individuals.

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Parallel Session 2: Metabolic Control of the Immune System

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Metabolic aspects underlying immune regulation

H. Waldmann, F. Regateiro, J. Ma, D. Howie, C. Peter, E. Adams, A. Kendal & S. Cobbold
Sir William Dunn School of Pathology, Oxford University, Oxford, UK

Dominant tolerance induced by therapeutic antibody blockade of T-cell function is dependent on TGFbeta signalling to T-cells, and on the recruitment/induction of FoxP3+ CD4 regulatory T-cells. TGFbeta is a potent upregulator of CD39 and CD73 on activated T-cells if unimpeded by proinflammatory cytokines. In conjunction with antigen, in circumstances where mTOR signaling is inhibited in T-cells, TGFbeta can promote the generation of FoxP3+ iTreg. There are a number of routes (such as nutrient sensing) to achieving mTOR inhibition, and these also, may play a synergistic part in promoting regulation. Evidence will be presented that regulation can operate within the tolerated tissue itself, by creating zones of immune privilege.

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Regulation of T cell differentiation and function by mTOR

J. Powell,* G. Delgoffe,† K. Pollizzi,‡ E. Heikamp‡ & A. Waickman‡
**Oncology, Johns Hopkins University, Baltimore, MD, USA, †St. Jude Children's Research Hospital, Memphis, TN, USA, ‡Johns Hopkins University, Baltimore, MD, USA*

The integration of multiple cues in the immune microenvironment determines the outcome of TCR engagement. The mammalian Target of Rapamycin (mTOR) is a PI3-kinase family member that is critical for integrating environmental cues. Downstream mTOR signaling is facilitated by two distinct signaling complexes (mTORC1 and mTORC2) whose activation is differentially regulated. We hypothesized that this evolutionarily conserved kinase might play a role in integrating cues from the immune microenvironment. Indeed, T cells lacking mTOR not only fail to become effector cells but become Foxp3+ regulatory T cells even under normally activating conditions. Next we created mice with specific deletions in downstream mTOR signaling pathways. Experiments employing such mice revealed that Th1 and Th17 differentiation is selectively regulated by Rheb dependent mTORC1 signaling. Likewise, Rheb dependent mTORC1 signaling was required for the generation of CD8+ effector function. Indeed, T cells with hyper-active mTORC1activity (TSC2-/- T cells) differentiated into superior effector cells. In contrast, by deleting rictor, a critical component of mTORC2 signaling, we have revealed an essential role for mTORC2 in regulating Th2 responses. Interestingly mTORC2 deficient CD8+ T cells developed both robust effector function and increased memory generation. Finally, our studies revealed that regulatory T cell generation is facilitated only in the setting of inhibiting both mTORC1 and mTORC2. Overall, our findings form the basis of a novel paradigm whereby mTOR integrates environmental cues to dictate the outcome of antigen recognition.

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Interplay between the external environment and immune cells

M. Veldhoen
Lymphocyte Signalling and Development, Babraham Institute, Cambridge, UK

The transcription factor aryl hydrocarbon receptor (AhR) is selectively expressed in some immune cells, importantly in both Th17 and gamma/delta T cells. Its activation is important for optimal development of the Th17 cell subset and results in enhanced activation of both Th17 and IL-17 producing gamma/delta T cells. Immune protection against invading microorganisms starts at epithelial barrier sites. At these sites reside specialised intra-epithelial lymphocytes (IELs) that are important not only as a first line of defence but also in epithelial barrier organisation and wound repair. Especially at epithelial sites, AhR activation in response to endogenous and exogenous ligands may constitute a way in which environmental stimuli could affect the immune status. New data regarding the role of such stimulation will be discussed.

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Arginine availability: a crucial checkpoint in the adaptive immune response

M. Munder
Third Department of Medicine, Hematology, Oncology, and Pneumology, University Medical Center Mainz, Mainz, Germany

Arginine depletion by arginase-expressing myeloid immune cells profoundly modulates T lymphocyte functions and is crucially involved in the regulation of the immune response during infection, inflammatory diseases and tumor growth. While T cell proliferation is completely shut down upon arginine withdrawal, synthesis of only a subset of cytokines (e.g. IFN-χ) is equally inhibited, while secretion of other cytokines (e.g. IL-2) is largely independent of extracellular arginine. Other important T cell functions, like CD8+ T cell mediated cytotoxicity, are completely preserved in the absence of arginine. By proteomic analysis we detected an impaired dephosphorylation of the actin-binding protein cofilin upon T cell activation in the absence of arginine. We show that this correlates with impaired formation of the immunological synapse between T cells and antigen presenting cells. Further downstream, MEK and PI3K activity are reciprocally regulated in association with impaired cofilin dephosphorylation. Phosphorylation of the stress response kinase GCN2 is a sensor of T cell arginine depletion in human T cells. Finally, arginine deficiency can induce human primary T cells to express the enzyme argininosuccinate-synthase (ASS) de novo. Upon exogenous application of citrulline T cells can then synthesize their own endogenous arginine via rate limiting ASS with consecutive reconstitution of T cell functions. This citrulline-mediated reconstitution of adaptive immune functions in the context of arginase-mediated arginine deficiency is a promising therapeutic strategy to boost the immunological anti-tumor response. In summary, our data unravel a complex biochemical and functional reprogramming of human T cells under arginine limiting conditions.

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Mitochondrial respiratory capacity is a critical regulator of CD8 T cell memory development

G. J. van der Windt, B. Everts, C.-H. Chang, J. Curtis, T. Freitas, E. Amiel, E. J. Pearce & E. L. Pearce
Trudeau Institute, Saranac Lake, NY, USA

CD8 T cells undergo major metabolic changes upon activation, but how metabolism influences the establishment of long-lived memory T (T_M) cells after infection remains a key question. We found that CD8 T_M cells, but not effector CD8 (T_E) cells, possess substantial mitochondrial spare respiratory capacity (SRC). SRC is the extra capacity available in cells to produce energy in response to increased stress or work and as such is associated with cellular survival. We show that IL-15, a cytokine critical for CD8 T_M cells, regulates SRC and oxidative metabolism by promoting mitochondrial biogenesis and expression of carnitine palmitoyl transferase (CPT1a), a metabolic enzyme that controls the rate-limiting step to mitochondrial fatty acid oxidation (FAO). Our findings highlight an essential role for mitochondria in regulating the T cell survival and function after infection, and suggest that drugs that target mitochondrial SRC could hold promise as immunotherapeutics and might warrant further study for their ability to alter T cell responses.

Parallel Session 3: Mathematical Modelling in Immunology

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Revealing unexpected cellular dynamics during T cell homeostasis using mathematical models

I. Bains,* C. M. Brauner,† R. Callard,‡ D. Commenges,§ T. Hogan,¶ B. Seddon,¶ A. Shuvaev,§ C. Sinclair,¶ R. Thiebaut§ & A. Yates*
**Albert Einstein College of Medicine, New York, NY, USA, †Institut de Mathematiques, Universite Bordeaux 1, Bordeaux, France, ‡Institute of Child Health, University College London, London, UK, §INSERM U 907, Bordeaux 2 University, Bordeaux, France, ¶Division of Immune Cell Biology, MRC National Institute for Medical Research, London, UK*

The size and composition T cell compartment of the adaptive immune system is subject to strict homeostatic regulation. Homeostasis is achieved by careful orchestration of lymphocyte production, survival and cell division. While many of the molecular mechanisms governing homeostasis have been defined qualitatively, it is less clear at a quantitative level how these different mechanisms combine to achieve the homeostasis observed within the T cell compartment. This talk will present our latest results using mathematical modeling tools to understand lymphocyte dynamics required for normal T cell homeostasis. By developing models that describe lymphocyte behavior together with using experimental data to identify the key parameters of that behaviour, the models can then be used to make novel biological predictions that can be validated experimentally. We have used such modeling tools to understand the dynamics of positive selection during T cell development in the thymus and to investigate the cellular programme that underlies homeostatic proliferative responses of peripheral T cells in lymphopenia. In both cases, models predicted unexpected cellular behaviour and function that was subsequently validated by experimental investigation. The key findings of these studies will be presented.

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I know it's only mathematical modelling, but I like it, like it, yes I do

E. Palmer, D. Naeher, C. King, B. Hausmann & S. Koehli
Transplantation Immunology, University Hospital-Basel, Basel, Switzerland

T cell tolerance is established in the thymus (central tolerance) and maintained in the body (peripheral tolerance). In both compartments, developing thymocytes or peripheral T cells are able to measure and 'interpret' antigen affinity, allowing them to make an appropriate response. The lecture will try to explain some elements of the principle of affinity, the initiation of TCR signaling and the cellular mechanisms required for a differentiated T cell response, using a combination of experimental data and very basic mathmatical modeling.

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Incorporating differentiation decisions into models of immune regulation using results from video microscopy

P. D. Hodgkin,* K. R. Duffy,† C. J. Wellard,* J. F. Markham,‡ J. Zhou,* E. D. Hawkins,§ J. Hasbold,* M. R. Dowling* & R. Holmberg*
**Immunology, Walter and Eliza Hall Institute, Parkville, Vic., Australia, †Hamilton Institute, Maynooth, Ireland, ‡National ICT Australia, University of Melbourne, Parkville, Australia, §Cancer Immunology Program, Peter MacCallum Cancer Institute, East Melbourne, Vic, Australia*

Stimulated B cells undergo a mix of proliferation, isotype switching, differentiation to plasma cells, and apoptosis to generate a remarkable level of cellular heterogeneity within a few days. We can list three classes of competing explanation for the appearance of different cell types:

1 External control directs cells to different fates;
2 Asymmetric cell divisions give rise to cells with different fate lineages;
3 Internal stochastic processes foster the allocation of alternative fates.

We used time lapse *in vitro* microscopy of B lymphocytes stimulated with anti-CD40 and IL-4 to directly measure time from division to a series of different fates for thousands of individual cells. We find the times taken to isotype switch, to develop into a plasmablast, to divide and to die are all highly variable within the population. However, there are strong correlations between the fate of sibling cells, with little evidence for asymmetric fate allocations. We find that the data are consistent with the hypothesis that each cell possesses stochastically independent processes for each distinct fate. Mathematical models built on rules drawn from single cells can recreate the quantitative behaviour of B cell populations. Thus, we propose that stochastic processes underlie, to a surprisingly large extent, the remarkable cellular heterogeneity that is a feature of the immune system.

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Burst-like responses of activated T lymphocytes: role in immune protection and in the pathogenesis of HIV infection

Z. Grossman[*,†] **& G. Bocharov**[‡]

[*]*Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD, USA,* [†]*Department of Physiology and Pharmacology, Tel Aviv University, Tel Aviv, Israel,* [‡]*Institute of Numerical Mathematics, Russian Academy of Sciences, Moscow, Russia*

Burst-like proliferation is a hallmark of antigen-specific immune responses but 'spontaneously' arising proliferation bursts represent a significant minority of dividing CD4 T cells in unperturbed mice. The mechanisms regulating clonal expansion and contraction remain unclear. A log-linear relation was found between CD4 T-cell precursor number (PN) and factor of expansion. A parsimonious mathematical model with feedback-regulated proliferation versus differentiation rates reproduced this kinetics. Accordingly, differentiated effectors limit the growth of precursors, locally, by increasing their differentiation rate in a dose-dependent manner. Consequently, expansion is reversed after a delay that depends on initial PN, accounting for the dependence of the peak on that number. The analysis highlighted the 'overshooting' property of the immune response, instrumental for effective protection. Differentiation of a minority of responding T cells is arrested at the central-memory stage. This arrest may depend on 'tuning', the induction of a reversible increase in the activation-threshold, linked to increasing numbers of activated cells that interact with APC.

In HIV infection, activation bursts dominate T-cell turnover. High concentrations of pro-inflammatory agents broadly augment the nominally weak interactions among T cells and self peptide-MHC ligands so that the resulting stimuli exceed both the T-cell activation threshold and the 'break-through' T cell-APC interaction threshold required for activation bursts. Activation and HIV transmission are proximal events. Coupled proliferation-infection bursts are initiated by latently infected CD4+ T cells and lead to the regeneration of those. Deciphering the interconnected dynamics of HIV-induced inflammation, immune activation and infected-cell regeneration has contributed to our understanding of HIV pathogenesis.

Parallel Session 4: In Control: PAMPs, DAMPs and RAMPs

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Toll-like receptors and NOD-like receptors: key drivers of innate immunity and inflammation

L. O'Neill
Trinity Biomedical Sciences Institute, School of Biochemistry and Immunology, Dublin, Ireland

In the field of inflammation research, the most important advances in the past 10 years has been in the uncovering of multiple pathways involved in innate immunity. The best characterised involve the Toll-like receptors (TLRs) and NOD-like receptors (NLRs). Work on knockout mice and the use of inhibitors continues to validate proteins in these systems in disease. From work on Nlrp3 there has also been a resurgance of interest in the IL1 system as a key driver of inflammation in diseases such as gout and diabetes (both Type I and Type II). For investigators interested in signal transduction, the area has proved very fruitful in terms of the discovery of new signalling pathways and processes. We now have a good understanding of the major components activated by TLRs, notably the TIR domain − containing adapters that initiate signalling following recruitment to TIR domains within the TLRs themselves, the IRAK family of protein kinases that are then recruited, and a series of ubiquitination and phosphorylation reactions that ultimately lead to the activation of transcription factors such as NF-kappaB and IRF family members. A role for metabolic processes including glycolysis in the regulation of signalling is also an emerging theme, suggesting that restoration of homeostasis after tissue injury and infection is a key goal of innate immunity. As we continue to unravel the molecular details of these processes, new therapeutic options will present themselves.

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Prokaryotic molecular chaperones: has convergent evolution turned moonlighting PAMPs into DAMPs?

B. Henderson
UCL − Eastman Dental Institute, London, UK

Among the growing number of circulating human molecules termed Damage-Associated Molecular Pattern (DAMPs) are the molecular chaperones and protein-folding catalysts that function, intra- and extra-cellularly to control protein folding. It is now recognised that many of these proteins are secreted by cells and are powerful immune regulators, either endogenously, or through the ability to bind and present peptides to T lymphocytes. Prokaryotes also require a range of molecular chaperones and protein-folding catalysts for their survival and their essentiality suggests that they are examples of Janeway's PAMPs. A number of these prokaryotic proteins are secreted and interact with the human immune system, having particular influence on macrophages and dendritic cells. The potential moonlighting roles of bacterial molecular chaperones and protein-folding catalysts in bacterial infection will be described and evidence for the hypothesis that the moonlighting actions of these proteins as DAMPs is an example of convergent evolution will be presented. This raises the possibility that the large number of bacteria resident in the human, through release of molecular chaperones and protein-folding catalysts, could interact with the signalling properties of human DAMP receptors and thus interfere with normal immune homeostasis.

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HSP's and immunotherapy

B. Prakken, E. Zonneveld, Y. Vercoulen & F. van Wijk
University Medical Center Utrecht, Utrecht, The Netherlands

Current therapies of human autoimmune diseases such as Juvenile Idiopathic Arthritis are based on non-specific immune suppression (1). As these therapies do not cure the disease, lifelong treatment is necessary with increased risks of serious side effects. Alternative strategies based on immune deviation instead of suppression and on antigen specific instead of non-specific targeted are therefore crucial. We previously showed that antigen specific targeting of a stress related bystander antigen, heat shock protein 60 (HSP60) is a promising alternative to current strategies (2). Though translation from bench to bedside is very difficult in this field we have been able to make substantial progress first by identifying pan-DR binding epitopes (3), and more recently by substantiating their anti-arthritis potential in a model of arthritis (4)

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Parallel Session 5: Adoptive Cell Therapy for Immuno-Related Diseases

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Translation of regulatory T cells into the clinic: preclinical and clinical studies

B. Blazar
University of Minnesota, Minneapolis, MN, USA

This presentation will discuss our phase I dose escalation clinical trial of umbilical cord blood (UCB) Treg infusion which included 23 patients who received a double UCB transplant and nTreg expanded from a third UCB unit. Data from a xenogeneic model of graft-versus-host disease (GVHD) showed that Treg efficacy correlated with the number of cells remaining on day 10−14, so the next cohort received on day 14 a second dose of Treg, cryopreserved at the time of initial Treg transfer. No infusional toxicity was observed; nor was there an increased risk of infection, relapse or early mortality. Treg reduced the incidence of grade II-IV acute GVHD (43% versus 61%, $P = 0.05$) compared to 108 historical controls treated identically except for Treg. Five of 23 patients did not receive the targeted dose.

Therefore, we have explored:

1 Approaches to increase Treg potency in the face of limited Treg numbers.
2 Approaches to dramatically improve Treg yield.
3 Alternative Treg sources, including inducible Tregs. The clinical trial results and approaches to improve outcomes using Tregs will be discussed.

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Regulatory T cell therapy to treat auto and alloimmunity

J. Bluestone,* M. Yadav,* S. Bailey-Bucktrout,* S. McClymont,* A. Putnam,* M. Lee,* W. Liu* & Q. Tang*,†
**UCSF Diabetes Center, San Francisco, CA, USA, †Department of Surgery, UCSF, San Francisco, CA, USA*

Regulatory T cells (Tregs) are critical in maintaining immune system homeostasis and tolerance to self-antigens. Tregs arise both in the thymus (called natural Tregs, nTregs) and in the peripheral immune system (called adaptive Tregs, aTregs). A defect in functions of Tregs results in the onset of autoimmune diseases both in humans and mouse while adoptive transfer of Treg cell populations suppress autoimmunity and transplant rejection. Thus, there has been increasing efforts to translate the animal adoptive cell-based therapies to the treatment of a variety of diseases. However, the basis for Treg defects and specific roles of nTreg cells and aTreg cells on tolerance and inflammation and their relative contributions in autoimmunity are not fully understood. This presentation will focus on the role of Treg subset stability and function in autoimmunity. The presentation will also highlight the potential for Tregs in therapeutic applications.

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Tr1 cells in allogeneic transplantations

R. Bachetta,* S. Gregori,* B. Lucarelli,* M. Battaglia,† F. Ciceri‡ & M. Grazia Roncarolo*,§
**Department of Regenerative Medicine, Stem Cells and Gene Therapy, San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET), Milan, Italy, †San Raffaele Diabetic Research Institute, Milan, Italy, ‡Hematology and BMT Unit, San Raffaele Scientific Institute, Milan, Italy, §Vita Salute San Raffaele University, Milan, Italy*

Type 1 regulatory T cells are an inducible subset of regulatory T cells. They develop in the periphery upon chronic antigen-stimulation in the presence of IL-10 produced by tolerogenic antigen-presenting cells (DC-10). The immuno-modulatory activities of Tr1 cells reside in their ability to secrete IL-10 but also in their property of cell-to-cell contact-dependent killing of target myeloid cells mediated by granzyme B and perforin. Tr1 cells are distinct from nTr cells since they are independent from FOXP3 expression for both their function and generation. In preclinical models of islet transplant we demonstrated that adoptive transfer of alloAg-specific Tr1 cells, but not polyclonal Tr1 cells, promote tolerance. In humans we showed that both exogenous IL-10 or IL-10-derived from tolerogenic DC can be used to generate alloAg-specific Tr1 cells *in vitro* suitable for cell therapy. Moreover, Tr1-like cells can be generated by transducing human $CD4^+$ T cells with a lentiviral vector encoding for IL-10.

Clinical studies in hematopoietic stem cell transplantation using alloAg-specific Tr1-based cell therapy demonstrated that infusion of donor-derived host-specific Tr1 cells is feasible and well tolerated, since no acute adverse effects related to the infusion were observed, whereas rapid and long-term immune-reconstitution together with absence of relapse were achieved. However, the fate of the infused Tr1 cells in an inflammatory environment and the interaction with pharmacological agents remain to be evaluated. Follow-up studies in larger patients cohort are desired to broaden the use of Tr1-cell based therapy to other transplantation settings and T-cell mediated diseases including autoimmunity and allergy.

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$CD4^+CD25^+$ regulatory T cells in allogeneic stem cell transplantation

M. Edinger

Department of Hematology and Oncology, University Hospital Regensburg, Regensburg, Germany

Graft-versus-host disease (GVHD) is a major complication after allogeneic stem cell transplantation (SCT) and induced by donor T cells. After their activation and expansion, such alloreactive T cells attack typical target organs, such as skin, liver and gut. An important research goal in SCT is the separation of beneficial donor T cell effects, such as their graft-versus-leukemia/lymphoma response, from harmful effects, such as severe GVHD. In murine disease models, we previously showed that the adoptive transfer of donor $CD4^+CD25^+$ Treg does not induce GVHD, but protects from GVHD otherwise induced by co-transplanted conventional T cells. Importantly, donor Treg do not paralyse donor T cell functions, as their graft-versus leukaemia/lymphoma activity can be maintained in the presence of Treg. Thus, the adoptive Treg transfer seems an attractive strategy for the prevention of GVHD in humans. We previously described methods for the GMP-compatible isolation and expansion of human Treg. Furthermore, we showed that only $CD45RA^+$ Treg cells generate homogeneous Treg cell lines after *in vitro* expansion, while even CD127-depleted $CD4^+CD25^+$ T cells partially lose their Treg cell characteristics, as illustrated by the loss of FOXP3 expression, the emergence of cytokine producers and by changes in the methylation pattern within the FOXP3 locus. Treg that lost FOXP3 expression upon expansion showed a switch towards a Th2-like differentiation pattern. Based on these findings, we suggest that the isolation and expansion of naïve Treg cells is the safest strategy for clinical trials in SCT exploring their suppressive activity for GVHD prevention or therapy.

Parallel Session 6: B Cells, Antibody and the Control of Infectious Disease

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Mirna-155 regulates B cell function

E. Vigorito
Babraham Institute, Cambridge, UK

The differentiation programme of immune cells in the course of an immune response is traditionally viewed in terms of regulation by cytokines and transcription factors. However, studies in the past few years have shed light on the role of post-transcriptional regulation of gene expression by miRNAs. In animals, each miRNA is predicted to have up to hundred target genes and, conversely, many genes can be simultaneously targeted by several miRNAs. One of the main functions proposed for miRNAs is often referred as 'fine tuning' expression of protein coding genes or adjusting the mean expression level of target genes. Work conducted in my lab has focussed on understanding the role of one miRNA, miR-155, in regulating B cell function. In this presentation I will provide some evidence indicating that subtle changes in gene expression by miRNAs can result in profound phenotypic alterations.

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B cell TLRs and the antibody response to virus particles

A. DeFranco,* B. Hou,† P. Saudan,‡ G. Ott,§ R. L. Coffman§ & M. Bachmann‡
**Microbiology and Immunology, UCSF, San Franicisco, CA, USA, † Institute of Biophysics, Chinese Academy of Sciences, Beijing, China, ‡Cytos Biotechnology AG, Zurich-Schlieren, Switzerland, §Dynavax Technologies, Berkeley, CA, USA*

The contribution of Toll-like receptor (TLR) signaling to T cell-dependent (TD) antibody responses was assessed by using mice lacking the TLR signaling adaptor MyD88 in individual cell types. When a soluble TLR9 ligand was used as adjuvant for a protein antigen, MyD88 was required in dendritic cells but not in B cells to enhance the TD antibody response, regardless of the inherent immunogenicity of the antigen. In contrast, a TLR9 ligand contained within a virus-like particle (VLP) substantially augmented the TD germinal center IgG antibody response, and this augmentation required B cell MyD88. Inactivated influenza virus particles behaved similarly. When a soluble allergen (Fel d1) was conjugated to the VLP, it acquired the ability to engage B cell MyD88 to boost the anti-Fel d1 IgG response, and there was a trend toward greater enhancement with higher epitope density on the VLP, suggesting that strong BCR signaling is required to enable this mechanism of germinal center response. We have also examined the cell type requirements of MyD88 for lupus-like autoantibody production in Lyn-/- mice and found that B cell MyD88 is important for this response. The ability of B cells to discriminate between antigens based the physical form of a TLR ligand likely reflects an adaptation to facilitate strong anti-viral antibody responses. In addition, this mechanism of B cell activation may inadvertently contribute to lupus-like autoimmunity.

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B1b lymphocytes occupy a unique functional niche in anti-bacterial immunity

K. Alugupalli
Department of Microbiology and Immunology, Thomas Jefferson University, Philadelphia, PA, USA

Infectious diseases are the leading cause of death worldwide and vaccination is the most effective means to control them. A hallmark of the adaptive immune system is the generation of B cell memory, which provides a long-lasting protective antibody response that is central to the concept of vaccination. Studies in the murine system revealed a distinct function for B1b lymphocytes, a minor subset of mature B cells that closely resembles that of memory B cells in a number of aspects. In contrast to the development of conventional B cell memory, the development of B1b cell-mediated long-lasting antibody responses occurs independent of T cell-help. T cell-independent (TI) antigens are important virulence factors expressed by a number of bacterial pathogens. TI antigens cannot be processed and presented to T cells and therefore are known to possess restricted T cell-dependent (TD) immunogenicity. Nevertheless, specific recognition of a variety of TI antigens by B1b cells and the highly protective antibody responses mounted by them clearly indicate a crucial role for this subset of B cells in the host's ability to overcome the restricted TD antibody response. The discovery of putative B1b cells in humans as well as in human hematopoeitic stem cell-engrafted mice and the importance of B1b cells in protection against human pathogens clearly indicate that studying the B1b cell ontogeny, homeostasis and function in murine models is directly relevant to humans. Understanding the mechanisms of long-term immunity conferred by B1b cells may lead to novel vaccination strategies.

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T cell-independent anti-pneumococcal B cell memory: the role of long-lived plasma cells

T. Defrance
INSERM U851, INSERM, Lyon, France

Thymus-independent (TI) responses have received relatively little attention because they display limited Ig isotype regulation, weak affinity and are thought not to confer long-term protective immunity. Nevertheless, the prejudice against TI responses begins to be raised thanks to a series of recent studies. The groups of R. Gerstein and M. Nussenzweig have demonstrated that TI Ag do generate memory B lymphocytes that can be assigned to the B-1b cell subset in the mouse. However contribution of these cells to the vaccine-induced humoral protection remains elusive due to the stringent negative control that is exerted on their activation by IgG antibodies. We have documented that a *S. pneumoniae* capsular polysaccharide can generate long-lived bone marrow plasma cells that are sufficient to confer full humoral immunity to the hosts. Our data indicate that while the pneumococcal capsular polysaccharide is by itself poorly immunogenic, it can generate long-lasting protective immunity both in adult and young mice when it is appropriately combined with a TLR agonist.

Plenary Session 1: Dendritic Cells

788
Fate mapping mononuclear phagocytes

S. Yona,* K.-W. Kim† & S. Jung*
**Immunology, Rehovot, Israel, †Weizmann Institute of Science, Rehovot, Israel*

While the last decade yielded major advances in our understanding of the mononuclear phagocyte system, many of its developmental and functional aspects remain poorly understood. Compared to the study of B and T cells, which profits from well-defined promoter/enhancer elements that allow *Cre-lox* mediated gene ablations, mononuclear phagocytes, and in particular myeloid progenitors have remained largely refractory to such approaches. Moreover, the exceeding short half-life of monocytes and classical dendritic cells represent an additional challenge to conditional genetic manipulations. Here, we will report our efforts to exploit the activity of the CX3CR1 promoter to target the system. Specifically, I will discuss recent insights into the fate of primitive and definitive hematopoetic mononuclear phagocyte precursors to peripheral macrophage and dendritic cells compartments, as well as monocyte dynamics gained from mice expressing constitutive or conditionally active Cre recombinase (CX3CR1-Cre and CX3CR1-CreERT2 mice).

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Sec22b controls the recruitment of endoplasmic reticulum to phagosomes in dendritic cells: functional consequences for cross presentation and phagosome maturation

S. Amigorena
Institut Curie U932, Paris, France

The role of the endoplasmic reticulum (ER) in phagocytosis and in antigen cross presentation has been a matter of sustained debate, at least in part because the molecular mechanisms underlying the proposed interactions between the two compartments were unclear. We now show that, in dendritic cells (DCs), the knock down of the ER-SNARE Sec22b − a member of a wide family of proteins involved in intracellular membrane fusion − inhibits the recruitment of ER resident proteins to phagosomes and to the *Toxoplasma gondii* vacuole. In Sec22b knocked down cells, the cross presentation of ovalbumin (OVA) coated on latex beads, of soluble OVA or of OVA expressed in *Toxoplasma gondii* was compromised. In contrast, MHC class II and endogenous MHC class I-restricted presentations of the same antigen were not affected. The knock down of Sec22b also inhibited the export of endosomal cargo to the cytosol and caused a marked increase of phagosomal proteolysis. We conclude that Sec22b plays a critical role in the recruitment of ER membrane proteins to phagosomes in dendritic cells and that ER recruitment modifies phagosomal functions to promote cross presentation.

Parallel Session 7: Inflammation, Immunity and Host Microbiome

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Immunomodulatory functions of segmented filamentous bacteria

A. Sczesnak,* N. Segata,† X. Qin,‡ J. F. Petrosino,‡,§ C. Huttenhower,¶ D. R. Littman* & I. Ivanov**
**Skirball Institute, New York University School of Medicine, New York, NY, USA, †Harvard School of Public Health, Boston, MA, USA, ‡Human Genome Sequencing Center, Houston, TX, USA, §Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX, USA, ¶Harvard School of Public Health, Boston, TX, USA, **Department of Microbiology and Immunology, Columbia University Medical Center, New York, NY, USA*

Commensal intestinal bacteria are indispensible for the proper functioning of the mammalian immune system. Both mucosal and systemic immune mechanisms are profoundly affected by the gut microbiota. Commensals provide not only essential immune protection against life-threatening infections, but shape pre-existing immune responses and thus modulate the ability of the host to respond to environmental challenges. These immunomodulatory functions are dependent on the composition of the commensal community and seem to be a property of individual members of this community. Investigation of the underlying mechanisms requires the identification of specific examples of such interactions. We have identified the murine commensal segmented filamentous bacteria (SFB) as capable of specifically shifting the homeostasis of effector T cell subsets in the small intestinal lamina propria. SFB induce preferentially Th17 cells and augment Th17 cell-dependent immune responses. As a first step toward the identification of immunomodulatory SFB products, we have sequenced the SFB genome and have performed initial analysis based on its annotation. This information will hopefully help the investigation of the mechanisms by which SFB affect intestinal immune responses and the development of new genetic tools for the study of the biology of this unique microorganism.

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Smad7, gut inflammation and cancer

M. Fantini
University of Rome 'Tor Vergata', Rome, Italy

Chronic inflammation is thought to be the leading cause of many human cancers. However defects of the immune system are associated to an increased risk to develop malignancies as shown by patients affected by congenital and acquired immune-deficiencies. Therefore the role of immune system in cancer development is still an open is issue.

Transforming Growth Factor (TGF)-beta plays a pivotal role in the control of the immune system activation and defects of the TGF-beta signaling in T cells cause spontaneous colitis. Accordingly, in human inflammatory bowel disease (IBD), characterized by an increased risk of colorectal cancer, chronic inflammation of the gut is sustained, at least in part, by the block of the TGF-beta signaling operated by the cytoplasmic molecule Smad7. Interestingly, while sustaining severe intestinal inflammation, high Smad7 resulted protective towards cancer development. Indeed mice overexpressing Smad7 in T cells developed more severe colitis but fewer tumors in the AOM/DSS model of colitis-associated colorectal cancer (CAC) as compared to wild type mice. Moreover, CAC development was associated with a downregulation of Smad7 in the lamina propria T cells of IBD patients. Inflammation in the Smad7 transgenic mice was characterized by the accumulation of Th1 cells and IFN-gamma-dependent increase of cytotoxic activity in the tumors mediated by CD8+ and NKT cells.

These data demonstrate that inflammation is not invariantly associated to an increased risk of cancer and Smad7 emerges as a unique factor able to uncouple these two phenomena.

Parallel Session 8: Molecular and Clinical Interfaces of Invariant Natural Killer T Cells (iNKT)

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Molecular basis of CD1d-iNKT receptor interactions

S. D. Gadola
Clinical and Experimental Sciences/Department of Rheumatology, University of Southampton Faculty of Medicine, Southampton, UK

During the past 15 years, a highly conserved T-lymphocyte subset of CD1d-restricted iNKT cells has been found to exert powerful regulatory functions at the innate-adaptive interface, thereby promoting either adjuvant or tolerogenic immune functions. The primary event in iNKT cell activation is the binding of their unique T cell receptor (iNKT TCR) to lipid antigen presenting CD1d proteins that are expressed on other cells. Many *in vivo* studies in diverse mouse models of cancer, infection and autoimmunity have raised hopes that therapeutic manipulation of iNKT cells, e.g. via activating lipid antigens, could be used to address important clinical unmet needs in humans. However, translation of these pre-clinical successes into human clinical trials has not yet been successful. Possible reasons for these difficulties include species differences in both iNKT cell repertoire and CD1d lipid antigen presentation in mice and humans. Recent evidence from our laboratory and other research groups that enhance our understanding of the molecular interface of human and mouse iNKT cells with CD1d support this hypothesis and thus may point towards refined iNKT-based strategies so that they don't end up as 'lost in translation'.

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CD1 lipid antigen presentation: self-antigen recognition by invariant TCRs translates microbial danger signals

M. Brenner,* P. Brennan,* R. Tatituri,* M. Brigl,* N. Cohen* & G. Besra†
**Harvard Medical School/Brigham and Women's Hospital, Boston, MA, USA, †University of Birmingham, Birmingham, UK*

CD1d-restricted T cells, called iNKT cells, are innate lymphocytes that bear an effector memory phenotype and stand poised to become activated rapidly. Identification of the lipid antigens that activate iNKTs is central to understanding the role of iNKT cells in inflammatory responses. Activation by microbial α-anomeric glycolipids occurs; however, data now indicates that iNKT cells are most often activated in situations where microbial foreign-lipid antigens are absent or not necessary. Instead, lipid self-antigens presented by CD1d regulate iNKT cell activation in the absence of cognate microbial foreign antigen recognition. This allows iNKT cells to respond to virtually all microbes as well as become activated in noninfectious inflammatory situations. Cytokines like IL-12 contribute significantly in such 'Innate, Cytokine and Self-Antigen-Driven iNKT Cell Activation'. We have identified potent endogenous b-anomeric linked monoglycosylated ceramide self-lipid antigens such as b-Glccosylceramide as important. This b-linked ceramide accumulates during infection and in response to Toll-like receptor (TLR) agonists. We propose that recognition of self b-GlcCer and other self-lipid antigens by the invariant TCR translate innate danger signals into iNKT cell activation in the absence of cognate recognition of microbial lipid-antigens. Self-antigen recognition and cytokine-driven activation now stand as essential activation features these mammalian innate T lymphocytes that underscore how they differ fundamentally from NK cells and from adaptive MHC-restricted T cells.

734
On/off TLR signaling decides pro-inflammatory or tolerogenic dendritic cell maturation upon cognate interaction with iNKT cells

M. Falcone
Immunology, Transplantation and Infectious Diseases, San Raffaele Scientific Institute, Milan, Italy

Invariant NKT cells (iNKT) participate in the innate immune response to promote anti-microbial and anti-tumor immunity but they are also crucial to maintain T cell tolerance and prevent autoimmune diseases. iNKT cells that differentially modulate adaptive immunity do not bear a unique phenotype and/or specific cytokine secretion profile thus opening questions on how a single T cell subset can exert opposite immunological functions. We performed co-culture experiments with immature myeloid DC and purified regulatory iNKT cells and found that under steady-state conditions, i.e. in the absence of any other maturation signal, iNKT cells triggered tolerogenic DC maturation. iNKT cell-modulated DC (nktDC) predominantly secreted IL-10 with minimal release of pro-inflammatory cytokines (IL-12, IL-6 and TNF-a) and showed important tolerogenic function, i.e., capacity to trigger differentiation of IL-10-secreting regulatory T cells and to induce peripheral tolerance towards pancreatic islets and prevent autoimmune diabetes. Our experiments in transwells with iNKT cells and knockout CD1d DC showed that iNKT cell-induced tolerogenic maturation of mDCs required cell-cell contact and engagement of the CD1d molecule on the DC surface.

In the presence of TLR-stimulation, the same iNKT cells exerted a strong adjuvant effect and sustained the proinflammatory DC maturation (up-regulation of the maturation markers and increased IL12p70 production).

Our data demonstrate that iNKT cells perform their dual roles through a single mechanism of action relying on the cognate interaction with myeloid dendritic cells (DCs) and leading to opposite effects depending on the presence of other maturation stimuli simultaneously acting on mDCs.

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NKT cell regulation of inflammatory B cell responses

S. L. Enoksson,* E. Grasset,* T. Hägglöf,* N. Mattsson,* S. Gabrielsson,* T. L. McGaha,† A. Scheynius* & M. Karlsson*
**Karolinska Institutet, Stockholm, Sweden, †Medical College of Georgia, Georgia, GA, USA*

Inflammation induced by inefficient clearance of dying cells can lead to autoimmunity. A hallmark of this inflammatory response is production of auto-antibodies by B cells that undergo affinity maturation and produce high affinity antibodies against self antigens. Thus, B cell activation has to be controlled during inflammatory stimulation to allow a balanced response. NKT cells have been shown to be able to increase B cell responses to foreign antigens but how these respond to inflammation in a autoimmunity setting is less studied. Here, we have applied to mouse models for autoinflammation by either injecting syngenic apopotic cells or the inflammatory cytokine IL-18. In both these models we find that B cells react with a low affinity self response that gives rise to little pathology. However, if NKT cells are depleted this response is greatly enhanced and autoreactive B cells are allowed to enter germinal centre and undergo affinity maturation. Thus, we have uncovered a mechanism where NKT cells limit the B cell response to self antigens under sterile inflammatory conditions.

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Therapeutic manipulation of iNKT cells in autoimmunity: preclinical models

J. Diana, L. Ghazarian, Y. Simoni, L. Beaudoin & A. Lehuen
INSERM U986, University Paris Descartes, Paris, France

Invariant natural killer T (iNKT) cells are regulatory T cells that can both inhibit autoimmune T cell responses and promote T cell responses to pathogens such as viruses. Since converging data in humans and mouse models suggest that viral infections influence the development of type 1 diabetes, we have investigated whether iNKT cells could prevent type 1 diabetes (T1D) during viral infections. iNKT cells are potent regulatory cells that inhibit the development of diabetes. Upon LCMV infection, iNKT cells promote distinct innate antiviral responses in the spleen and pancreas, leading respectively to enhanced or diminished adaptive CD8 T cell responses. iNKT cells induce tolerogenic properties of plasmacytoid dendritic cells (pDCs) in a tissue-specific manner. In the spleen, iNKT cells interact with conventional DC to favor adaptive anti-LCMV CD8 T cell responses. In contrast in the pancreas, iNKT cells promote the recruitment of pDC and stimulate their production of type 1 IFN. Few days later, pDCs migrate from the pancreas to the pancreatic lymph nodes where they produced large amount of TGF-beta inducing the conversion of Foxp3+CD4+ regulatory T (Treg) cells. These Treg cells migrate and accumulate in the pancreas where they produce TGF-beta dampening virus-specific and self-specific CD8 T cell responses, thereby preventing tissue damage and diabetes onset. This study reveals a fundamental cooperation between iNKT cells, pDCs and Treg cells during virus infection in the prevention of both virus-induced and spontaneous T1D. Therefore iNKT cells represent a promising cellular target for the prevention of T1D in humans.

Parallel Session 9: Immune Mechanisms in Atherosclerosis

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Artery tertiary lymphoid organs (ATLOs) organize autoimmunity in atherosclerosis and connect diseased arteries to the central nervous system

A. Habenicht,* S. Mohanta,* P. Srikakulapu,* V. Bontha,* L. Peng,* C. Yin,* D. Hu,* M. Beer,* F. Weih† & R. Grabner*
**Institute for Vascular Medicine, University of Jena, Germany, † Department of Immunology, Fritz Lipmann Institute, Jena, Germany*

Tertiary lymphoid organs (TLOs) emerge in adult organisms in response to unresolvable inflammation. ATLOs arise in the aorta adventitia of hyperlipidemic apoE-deficient mice adjacent to atherosclerotic plaques. We suggested that they may be involved in the control in autoimmune responses towards atherosclerosis. Though ATLOs contain all cell lineages to conduct T and B cell responses towards arterial wall-specific antigen(s), their functional impact remains unclear. TLOs are hallmarks and accelerators of autoimmune diseases. We therefore sought to examine the significance of ATLOs in atherosclerosis. We report that ATLOs:

1 Recruit naïve T cells into the arterial wall.
2 Convert $CD4^+/CD62L^+$ T cells into $CD4^+/CD62^-/FoxP3^+$ regulatory T cells.
3 Generate central memory and effector memory T cells.
4 Form B cell follicles and harbor B-1 cells and plasma cells.
5 Contain antigen-presenting cells ($CD11b^+/CD11c^{low}$, B-2 cells).

Thus, though hyperlipidemia is a systemic metabolic condition, T and B cell autoimmune responses in atherosclerosis are entirely restricted to atherosclerotic artery segments. ATLOs also engage in neuroimmune crosstalks with the nervous systems:

1 $NF200^+$ axon neogenesis occurs in adventitia segments adjacent to atherosclerotic plaques.
2 Prevertebral sympathetic ganglia are surrounded by T/B cell aggregates.
3 Spinal cord, choroid plexus, and distinct brain nuclei accumulate lipid and show breakdown of the blood brain barrier.
4 The adventitia is directly wired to brain nuclei that control cardiovascular functions as shown by transsynaptical pseudorabies virus tracing. Our data open new opportunities to understand atherosclerosis autoimmunity and indicate that the brain can sense and affect arteries through neuroimmune circuits.

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Bifunctional roles of complement and antibodies in atherosclerosis

D. Haskard, M. Talat, M. Lewis, V. Leung, S. Yun, J. J. Boyle & M. Botto
Imperial College, London, UK

Atherosclerosis is now widely seen as a chronic inflammatory disease driven by the response of macrophages to low density lipoproteins (LDL) deposited within the arterial wall. Our studies have focused on the pathophysiological contributions of humoral immune system to atherosclerosis in the LDL receptor deficient ($ldlr^{-/-}$) mouse model. $Ldlr^{-/-}$ mice fed a low fat diet to mimic a low grade atherosclerotic drive had accelerated atherosclerosis when crossed with C1q deficient mice (i.e. lacking a functional classical pathway) or with DAF or CD59 deficient mice (i.e. lacking endogenous inhibitors of complement pathway progression). Furthermore, atherosclerosis was also accelerated in $Ldlr^{-/-}$ mice deficient in serum IgM natural antibodies. Using recurrent injections of endotoxin to amplify disease progression, we found that acceleration of lesion formation was prevented in Factor B deficient mice lacking alternative pathway activity. Taken together, the data suggest that natural antibodies and classical pathway activation play a protective homeostatic role in atherosclerosis prevention, probably by enhancing the safe disposal of oxidised lipoproteins and other tissue debris. However activation of the alternative pathway, for examle by endotoxin, may overwhelm endogenous complement inhibitors and result in pathogenic effects related to the terminal pathway. The talk will apply these principles to a dissection of how atherosclerosis is accelerated in SLE.

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Monocyte and dendritic cell subsets in atherosclerosis

G. J. Randolph
Department of Pathology and Immunology, Washington University in St. Louis, St. Louis, MO, USA

Monocytes are well established to be key drivers of atherosclerotic plaque progression. More recently, roles for dendritic cells in atherosclerosis have been uncovered. Monocytes and dendritic cells are heterogeneous and can be divided up into discrete subpopulations. These subpopulations and recent advances in our understanding of their biological roles in atherosclerosis will be discussed. A major interest in my laboratory has been understanding whether monocytes that enter atherosclerotic plaques exhibit behaviors associated with dendritic cells, especially the ability to emigrate out of lesions to draining lymph nodes. In particular, we have wondered whether resolution of plaques (regression) requires that monocyte-derived emigrate out of the plaque environement. Our recent data indicate that contraction of monocyte-derived cells in plaques is mostly driven by cessation in moncyte entry and a steady rate of local death rather than egress. These data has caused to reconsider the role of lymphatic trafficking in resolution of inflammation in general and to study monocyte differentiation during inflammation in detail. Data will be presented to argue that lymphatic trafficking and dendritic cell differentiation play minor roles in resolution of acute and chronic inflammation, including but not limited to atherosclerotic plaque.

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Mechanisms of accelerated atherosclerosis

Z. Mallat
University Cambridge, Cambridge, UK

Atherosclerosis is a chronic inflammatory disease of the arterial wall responsible for most ischaemic cardiovascular diseases. Circulating levels of several cytokines are associated with disease burden, and CRP levels predict the risk of future cardiovascular events. Furthermore, the incidence of cardiovascular disease (CVD) is increased in patients with chronic systemic inflammatory diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). Intriguingly however, classic circulating inflammatory biomarkers are not related to atherosclerosis progression in immune-mediated inflammatory diseases (IMIDs), suggesting more subtle intricate links between atherosclerosis and IMIDs.

We have shown that subpopulations of regulatory T (Treg) cells potently inhibit atherosclerotic lesion development and inflammation. Our recent work indicates that defective clearance of apoptotic cells, a characteristic of SLE, impairs the regulatory immune response in atherosclerosis and accelerates lesion development and inflammation, suggesting an important interplay between innate and adaptive immunity in promoting lesion development and progression. On the other hand, we have recently reported that depletion of mature B cells using CD20 monoclonal antibody induces a significant reduction of atherosclerosis in various mouse models of atherosclerosis. B cell depletion diminished T cell-derived IFN-gamma secretion and enhanced production of IL-17; neutralization of the latter abrogated CD20 antibody-mediated atheroprotection. These results suggest that impairement of Treg cell function and activation of B cell responses may be important pathophysiological links between IMIDs and atherosclerosis progression.

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Immune cell dynamics and antigen presentation in experimental atherosclerosis

P. Maffia[*,†]
[*]*Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, UK,* [†]*Department of Experimental Pharmacology, University of Naples Federico II, Naples, Italy*

Atherosclerosis is associated with local T and B cell immune responses. Lymphocytes mainly reside in the adventitia of normal arteries with accelerated recruitment observed in the pathology. In aged apolipoprotein E-deficient ($apoE^{-/-}$) mouse abdominal aortae adventitiae, well structured artery tertiary lymphoid organs (ATLOs) harbour dendritic cells, T cells, germinal centers within B cell follicles, and plasma cells. However, the impact of ATLOs on atherosclerosis has not yet been addressed. By using multiphoton laser-scanning microscopy we demonstrate that following markedly accelerated recruitment, leukocytes in ATLOs show dramatically enhanced movement when compared to adventitial leukocytes in wild type (WT) mice. Indeed, cell dynamics in ATLOs resembled those in the lymph nodes of the same mice. No differences in transferred cell dynamics were observed between $apoE^{-/-}$ and WT mice lymph nodes. To further support the importance of the local vascular adaptive immune response, I will then discuss the capacity of antigen presenting cells (APCs) of presenting *in vivo* systemically administered antigen directly in the diseased vessels and will detail the kinetic of local versus systemic antigen presentation in atherosclerosis. Finally, I will focus on the role of a specific subset of APCs, the plasmacytoid dendritic cells (pDCs). We demonstrated that the aorta and spleen of both $apoE^{-/-}$ and WT mice displayed similar numbers of pDCs, with similar activation status. In contrast, only aortic pDCs in $apoE^{-/-}$ mice were capable of presenting *in vivo* systemically administered antigen. pDC depletion significantly reduced atherosclerosis formation in the aortic sinus leading to a more stable plaque phenotype.

Parallel Session 10: Senescence and Exhaustion in the Immune System

868
Signaling and homeostasis alterations in aged naïve T-cells

J. Nikolich-Zugich
Immunobiology, University of Arizona, Tucson, AZ, USA

Aging is characterized by a variable and incompletely understood loss of immune protection against infection. Changes associated with this susceptibility have been collectively called immunosenescence, but to this day we still do not know which of these defects carry key responsibility for increased susceptibility to infection. While age-related changes have been described in many components of immunity, the most consistent and the most profound ones have been found in T-cells and restoration of T-cell immunity was usually accompanied by improved immune function and immune defense. Naïve T-cells in the last third of life display a number of alterations that affect their responsiveness to acute infection and that manifest themselves in incomplete proliferation and abortive differentiation into effector T-cells. We have dissected that phenomenon to demonstrate that naïve T-cell precursors already exhibit changes imparted upon them by over-exuberant proliferation in response to homeostatic, life-promoting cytokines. We further demonstrate that transcriptional programming of such precursors with regard to the short-lived effector cells (SLEC) is compromised when infection is encountered. Relationships between, and correction of, these defects will be discussed in the context of our most recent data.

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Tim-3 in T cell senescence and exhaustion

V. Kuchroo & A. Anderson
Center for Neurologic Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

T cell immunoglobulin-3 (Tim-3) is a molecule expressed on both Th1 and T cytotoxic 1 (Tc1) cells but not on other Th or Tc subsets. Triggering of Tim-3 by its ligand, galectin-9, induces a death signal in Th1/Tc1 cells, thereby contracting Th1/Tc1 immune responses and preventing undesired immunopathology. Tim-3, therefore, is a molecule similar to CTLA-4 and PD-1 that are also expressed on T cells and serve to contract T cell responses. It has been noted that in chronic disease states, dysfunctional or exhausted T cells express immune checkpoint molecules. Thus far, PD-1 has been the hallmark immune check point molecule expressed on exhausted T cells. Now, Tim-3 expression has been found on exhausted T cells in both chronic viral infection and cancer in both mice and humans. We have found that Tim-3 is expressed on both $CD4^+$ and $CD8^+$ tumor-infiltrating lymphocytes (TILs) in multiple different solid tumors. Interestingly, all $CD8^+$ $Tim\text{-}3^+$ TILs co-express PD-1 and $Tim\text{-}3^+PD\text{-}1^+$ TILs represent a predominant fraction of the $CD8^+$ T cells infiltrating tumors. $CD8^+Tim\text{-}3^+PD\text{-}1^+$ TILs exhibit the most severe exhausted phenotype as defined by failure to proliferate, produce IL-2, TNFa and IFNg. We have further found that combined immunotherapeutic targeting of the Tim-3 and PD-1 pathways is more effective in controlling tumor growth and restoring T cell function than targeting either pathway alone. Collectively, our data support that combined targeting of the Tim-3 and PD-1 signaling pathways is highly effective in restoring anti-tumor immunity.

869
Stress and immunesenescence: a tale of two hormones

N. Arora-Duggal,* J. Upton,† A. Phillips† & J. Lord*
**Immunity and Infection, University of Birmingham, Birmingham, UK, †Sport and Exercise Sciences, University of Birmingham, Birmingham, UK*

Although ageing is a complex process, we now know much of what happens to the immuen system with age at the individual cell and whole system level. In contrast, our understanding of how these various age-related changes interact twith other changes to key body systems and thus lead to immune frailty is incomplete. Ageing is accompanied by a loss of function in both the innate and adaptive arms of immunity (Immunesenescence), an increase in the level of circulating pro-inflammatory cytokines (Inflammaging), but also a decline in adrenal androgen production (Adrenopause) whilst concurrently peripheral glucocorticoid availability increases. This lecture we review how these changes in combination increase the susceptibility of older adults to the adverse effects of physical and emotional stress, exacerbating the age-related decline in immune competence and exposing the older individual to increased risk of infections. The discussion will cover effects of stress on aged elements of the innate immune system (neutrophil) and describe new and unpublished data concerning the effects of ageing and stress upon regulatory B cell numbers and function.

Parallel Session 11: Antibody Therapy and Fc Receptors

795

Opposing roles of FcgRIIb in dictating therapeutic response of direct targeting and immunostimulatory mAb

M. Cragg
University of Southampton, Southampton, UK

Monoclonal antibodies (mAb) represent a growing class of 'block-buster' cancer drugs, led by established reagents such as rituximab, trastuzumab, and bevacizumab, and followed by new and exciting reagents such as the anti-CTLA-4 mAb, ipilumumab. Despite such success, we are only now starting to understand how these drugs work and therefore how they can be augmented for better clinical efficacy.

mAbs can be separated into two types; those which target tumours directly, such as rituximab and alemtuzumab, and those which operate indirectly, for example by targeting key molecules in the immune system, such as CD40, 4-1BB and OX40. Current evidence suggests that the direct-targeting mAb operate mainly by recruiting natural effectors, such as NK and myeloid cells, to destroy unwanted cells, whilst the so-called immunostimulatory reagents boost pre-existing cancer immunity. We have understood for some time that Fc gamma receptors (FcR) on these effectors are critical for the in-vivo efficacy of direct-targeting mAb. For example, rituximab and alemtuzumab bind to lymphoid tumors and trigger cytotoxicity by engaging activatory FcR on the effector cells. In contrast, therapy through these mAb is dampened when the inhibitory FcR, FcRIIb, is co-engaged. However, the FcR requirement for immunostimulatory mAb has until recently been less clear.

In this talk, data will be presented revealing novel ways in which FcR regulate the activity of therapeutic mAb with clear implications for subsequent design of future reagents.

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Intracellular immunity: viral neutralization inside infected cells

L. James
MRC Laboratory of Molecular Biology, Cambridge, UK

Protection against bacterial and viral pathogens by antibodies has always been thought to end at the cell surface. Once inside the cell, a pathogen was understood to be safe from humoral immunity. However, recent data from our lab has shown that antibodies can routinely enter cells attached to viral particles and mediate an intracellular immune response (1). Antibody-coated virions are detected inside the cell by means of an intracellular antibody receptor, TRIM21 (2), which directs their degradation by recruitment of the ubiquitin-proteasome system. This system provides a highly potent block to viral infection. The degradation mediated by TRIM21 is also extremely rapid and clears the cell of infection before de novo viral synthesis. These findings demonstrate that neutralization, like other antibody functions, can be mediated by an effector mechanism. Upregulation of TRIM21 by interferon increases the efficacy of neutralizing antibodies. Conversely, removal of TRIM21 renders potently neutralizing antibodies non-neutralizing. These discoveries reveal an important new aspect to antibody function and highlight the importance of intracellular immunity (3).

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The role of alemtuzumab in allogeneic stem cell transplantation

K. Peggs
UCL Cancer Institute, London, UK

The humanized anti-CD52 monoclonal antibody alemtuzumab has been widely investigated for its ability to reduce the risk of graft-versus-host disease (GVHD) following allogeneic stem cell transplantation, either when administered directly to the patient as part of the conditioning regimen, or when mixed with the graft prior to stem cell infusion. In this regard, its ability to mediate profound T cell depletion makes it amongst the most successful of prophylactic preventative strategies. The prolonged half-life following *in vivo* administration means that it can both suppress host immunity to reduce the risks of graft rejection, and mediate subsequent lysis of donor T cells to reduce GVHD. Its ability to deplete B cells and a proportion of antigen-presenting cells may also be important in its activity. Furthermore, the former may be relevant in limiting the incidence of post-transplant lymphoproliferative disorders, which are associated with T-cell depleting strategies. Its use in reduced intensity transplantation protocols has facilitated more widespread use in elderly patients, particularly when unrelated donors are used, and may make greater degrees of mismatch a viable proposition, thus expanding the potential donor pool available for transplantation. The corollary is that immune reconstitution is delayed, infective complications more common, and graft-versus-tumour activity potentially impaired, resulting in higher relapse incidences. Current strategies incorporating adoptive cellular therapies, both for specific pathogens such as cytomegalovirus and to try to prevent or treat relapsed disease using unmanipulated donor-lymphocytes are incrementally improving transplant outcomes. Furthermore, direct modulation of the tumour microenvironment may also be key to improved outcomes.

859

Fc-receptors and innate immune effector cells involved in IgG activity

F. Nimmerjahn
University of Erlangen, Erlangen, Germany

IgG antibodies are the primary mediators of protective humoral immunity against pathogens and have been used therapeutically for over a century. They were first used as antitoxins for the treatment of infectious diseases in the pre-antibiotic era. Today, hyperimmune sera from human donors recovering from infection with specific viruses, such as hepatitis B, cytomegalovirus, and varicella zoster, are used to provide protective immunity to susceptible populations. Moreover, tumor specific antibodies have been successfully used in human cancer therapy. Besides these protective activities, IgG autoantibodies are the principal mediators of autoimmune diseases such as immune thrombocytopenia (ITP), autoimmune hemolytic anemia (AHA), and systemic lupus erythematosus (SLE). In addition to this pro-inflammatory activity antibodies also are known to have an anti-inflammatory activity. If infused at high doses, IgG can effectively suppress autoimmune mediated inflammation (IVIG therapy). Recent evidence suggests that both the pro- and anti-inflammatory activity of IgG is regulated by the sugar side chain that is attached to the CH2-domain of all IgG subclasses. Subtle variations in the composition of this sugar moiety will either enhance or decrease the pro-inflammatory activity. The presentation will discuss which factors influence these opposing effects of IgG and which effector cells are involved in mediating IgG activity.

840
CD16 and NK cell activation

G. J. Weiner
Holden Comprehensive Cancer Center, University of Iowa, Iowa, IA, USA

NK cell mediated antibody-dependent cellular cytotoxicity (ADCC) involving FcγRIIIa (CD16) likely contributes to the clinical efficacy of rituximab and other anti-cancer monoclonal antibodies (mAb). *In vitro*, higher concentrations of mAb are needed to induce changes on NK cells from subjects with the lower affinity CD16 polymorphism [158(FF)] compared to the high affinity polymorphism [158(VF/VV)]. *In vivo*, blood was evaluated before and 4 h after the initial dose of rituximab in 21 lymphoma subjects. Rituximab induced NK activation and a drop in circulating NK cell percentage in subjects with the high but not the low affinity polymorphism. We also evaluated the relationship between rituximab-induced complement fixation, NK cell activation, and NK cell-mediated ADCC. Down-modulation of NK cell CD16 and NK-cell activation induced by rituximab-coated target cells was blocked by human serum but not heat inactivated serum. Further studies demonstrated C3b deposition inhibits the interaction between the rituximab Fc and NK-cell CD16, thereby limiting NK activation and ADCC. In a mouse lymphoma model of mAb therapy, depletion of C3 enhanced the efficacy of mAb therapy. We conclude that NK activation occurs within 4 h of rituximab infusion in subjects with the high affinity CD16 polymorphism, but not those with low affinity polymorphism. The C3b component of complement can inhibit the interaction mAb-coated target cells with NK cell CD16, thereby reducing NK cell activation and ADCC. Together, these studies suggest mAb with enhanced affinity for CD16, and a reduced ability to fix complement, should be evaluated further as therapeutic agents.

Parallel Session 12: Epigenetic Modifications in Differentiation/Dedifferentiation

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Analysis of pro-inflammatory gene activation and RNA processing by RNA-Seq of nascent transcripts

D. Bhatt, A. Pandya-Jones, D. Black & S. Smale
Microbiology, Immunology, and Molecular Genetics, UCLA, Los Angeles, CA, USA

Macrophages respond to inflammatory stimuli by orchestrating the activation of hundreds of genes in a defined temporal cascade, with diverse transcriptional and post-transcriptional mechanisms contributing to the regulatory network. We previously classified LPS-induced genes on the basis of their requirements for nucleosome remodeling, requirements for new protein synthesis, kinetics of activation, and promoter properties. We have now examined pro-inflammatory gene activation in greater depth by performing RNA-Seq with fractionated chromatin-associated, nucleoplasmic, and cytoplasmic transcripts. This experimental strategy allowed a global, high-resolution analysis of the transcriptional regulation of diverse clusters of co-expressed genes, with a direct comparison to transcript processing and the transit of inducible RNAs from the chromatin to the nucleoplasm and the cytoplasm. The results provide quantitative insights into the relationship between transcriptional dynamics and distinct promoter and chromatin properties, and led to unexpected insights into the regulation of RNA processing.

622

How cohesin regulates gene expression and differentiation in non-dividing mammalian cells

V. Seitan,* K. Tachibana,† B. Hao,‡ A. G. Fisher,* K. Nasmyth,† M. Krangel‡ & M. Merkenschlager*
**MRC Clinical Sciences Centre, Imperial College London, London, †Department of Biochemistry, University of Oxford, Oxford, UK, ‡Department of Immunology, Duke University Medical Center, Durham, NC, USA*

Cohesin-mediated sister chromatid cohesion is essential for chromosome segregation and post-replicative DNA repair. In addition, data from model organisms and patients with Cornelia de Lange syndrome suggest a role for cohesin in the regulation of gene expression. This has been rationalized by findings that cohesin is recruited to mammalian chromosomes by the insulator protein CTCF and components of the transcriptional machinery, and that cohesin knockdown perturbs long-range chromosomal interactions. We have taken a genetic approach to determine the impact of cohesin on mammalian cell lineage-specific and developmental stage-specific gene expression *in vivo*. Deletion of the cohesin locus *Rad21* from non-dividing mouse thymocytes led to a defective chromatin architecture at the T cell receptor alpha chain (*Tcra*) locus, where cohesin binding sites flank the TEA promoter and the Eα enhancer. Cohesin mediated long-range promoter-enhancer interactions, *Tcra* transcription, post-transcriptional histone modifications that recruit the recombination machinery and ultimately *Tcra* rearrangement. These findings firmly establish a cell division-independent role for cohesin in *Tcra* locus rearrangement and provide a comprehensive account of the mechanisms by which cohesin enables cellular differentiation in a well-characterised mammalian system.

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Non-coding RNA transcription and the immunoglobulin repertoire

A. L. Wood, L. S. Matheson, D. J. Bolland, I. Osuch, J. R. Silva Martins, M. J. Stubbington & A. E. Corcoran
Nuclear Dynamics Programme, Babraham Institute, Cambridge, UK

One of the biggest logistic challenges of the adaptive immune system is making hundreds of antigen receptor (AgR) genes accessible for V(D)J recombination in the correct lymphocyte at the correct developmental time-point. This goal is essential for generating lymphocytes with both diverse and monoclonal antigen receptor repertoires. It requires activation, movement and silencing of AgR loci by a coordinated network of elements, factors, and epigenetic modifications, that together make the AgR loci the ultimate paradigm of epigenetic regulation. These mechanisms hold the key to the point of no return in lineage differentiation and commitment. For example, immunoglobulin heavy chain (Igh) D to J recombination is common in T cells, but Igh V to DJ recombination never occurs. Rather, Igh V to DJ recombination marks the commitment of progenitors to the B cell lineage. Our particular interest is in the role of non-coding RNA transcription in this process. We were the first to discover that ncRNA transcription precedes stage-specific recombination of immunoglobulin loci, and have made mouse models to determine its function *in vivo*. Previous epigenetic studies have been hampered by the size and repetitiveness of the Ig loci. We are using next generation sequencing to survey ncRNA transcription and histone modifications at unprecedented depth and resolution, and to relate these processes directly to V(D)J recombination of individual genes in the immunoglobulin repertoire as never before. The emerging picture is of an exquisitely fine-tuned interplay of epigenetic activation and repression that underpins selection of Ig genes in the immunoglobulin repertoire.

Parallel Session 13: Inflammation and Immunoregulation from Waste Disposal

778
Exposure of phosphatidylserine for signaling

S. Nagata
Department of Medical Chemistry, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Phospholipids are asymmetrically distributed between the outer and inner leaflets of the plasma membrane. Choline-containing phospholipids (phosphatidylcholine [PC] and sphingomyelin) are located primarily in the outer leaflet, while amine-containing phospholipids [phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphoinositides (PIP), and phosphatidic acid] are restricted to the cytoplasmic leaflet. The asymmetrical distribution of phospholipids is believed to be important for tight packing of the membrane lipids to decrease membrane permeability to solute. The asymmetrical phospholipid distribution is disrupted in various occasions. For example, when blood platelets are activated by collagen or thrombin, they expose PS on their surface to provide the catalytic surface for the tenase and prothrombinase complexes for blood clotting. Cells undergoing apoptotic cell death expose PS, which functions as an 'eat me' signal for phagocytes. Two types of lipid transporters are involved in the distribution of phospholipids in plasma membranes. The ATP-dependent aminophospholipid translocases transport aminophospholipids from the extracellular leaflet to the cytoplasmic side. While, scramblases transport phospholipids bidirectionally in an ATP-independent, but Ca^{2+}-dependent manner, and reduce the asymmetry of the lipids in membranes. We recently identified a membrane protein (TMEM16F) carrying 8-transmembrane segments as a phospholipid scramblase, and found a patient of Scott Syndrome who suffers of bleeding disorder due to the dysfunction of platelets carries a mutation in the TMEM16F gene. TMEM16F is a member of the TMEM16F family that is comprised of 10 members. Here, I will discuss biochemical and physiological functions of the TMEM16 family, and the physiological roles of the asymmetrical distribution of phospholipids.

804
Complement and recognition/clearance of dying cells

M. Botto
Imperial College London, London, UK

There is overwhelming evidence that deficiency of classical pathway complement proteins causes the development of systemic lupus erythematosus (SLE) in humans and mice. Complement is implicated in the pathogenesis of SLE in several ways and may act as both friend and foe. It has been suggested that one of the main activities of the classical pathway is to promote the resolution of inflammation by enhancing the clearance and uptake of dying cells by macrophages. We have developed a series of murine models of complement deficiency and SLE and found that these mice develop a lupus-like disease and have an impaired clearance of apoptotic cells. We have observed a similar phagocytic defect in macrophages derived from C1q-deficient humans cultured in autologous serum. This defect was rectifiable with purified human C1q. Consistent with these findings, we have data showing that macrophages from two lupus-prone murine strains have an impaired phagocytosis of apoptotic cells when compared with two non-autoimmune strains. Collectively these data strongly support the hypothesis that deficiency in complement predisposes to the development of lupus through inefficient removal of potentially pathogenic apoptotic cell debris. However, impaired clearance of such cells is, on its own, insufficient to produce autoimmunity. The data available from knockout mice emphasize that susceptibility to an autoimmune disease might depend on many factors in addition to the defective removal of dying cells.

764
Infection and apoptosis as a combined inflammatory trigger

J. Magarian Blander
Department of Medicine, Immunology Institute, Mount Sinai School of Medicine, New York, NY, USA

Microbial pathogens can initiate mitochondrial outer membrane permeabilization (MOMP) in host cells and as such trigger the mitochondrial pathway of apoptosis. Innate immune recognition of cells dying in this way by infection-induced apoptosis would involve recognition of ligands derived from the apoptotic host cell simultaneously with those derived from the infecting pathogen. We have shown that this scenario directs dendritic cells to concomitantly synthesize transforming growth factor β (TGF-β) and interleukin (IL)-6, two cytokines that subsequently favor the differentiation of naïve CD4 T cells into T helper 17 (T_H17) cells. *Citrobacter rodentium* is one rodent pathogen that targets mitochondria and induces apoptosis. We found that blockade of apoptosis during enteric *Citrobacter* infection impairs the characteristic T_H17 response in the intestinal lamina propria. Here, I will discuss these original findings and their implications.

595
The importance of natural IgM: scavenger, protector and regulator

M. Ehrenstein
University College London, London, UK

The existence of IgM has been known for more than a century but its importance in immunity and autoimmunity continues to emerge. Studies of mice deficient in secreted IgM have provided unexpected insights into its role in several diverse processes, from B cell survival to atherosclerosis as well as autoimmunity and protection against infection. Among a number of distinct properties that underlie its actions two stand out: its polyreactivity and its ability to facilitate the engulfment of apoptotic cells. Apoptotic cells can suppress inflammatory arthritis via the induction of IL-10 secreting B cells. Natural IgM is required for this protective effect which is linked to rapid clearance of apoptotic cells from the spleen. New B cell-targeted therapies for the treatment of autoimmunity have been shown to cause a reduction in serum IgM, potentially disrupting the function of this immunoregulatory molecule and increasing the susceptibility to infection.

Plenary Session 2: T Cell Plasticity

866
Reprogramming of Th2 cells into a stable GATA-3^{+}T-bet^{+} 'Th2 + 1' hybrid cell subset

M. Löhning
Department of Rheumatology and Clinical Immunology, Charité-University of Medicine, Berlin, Germany

Antigen and cytokine signals differentiate naïve T helper cells into distinct effector subsets, including classic Th1 and Th2 cells governed by the lineage-specifying transcription factors T-bet and GATA-3, respectively. We addressed the developmental fate of Th1 and Th2 effectors and challenged the commitment of Th2 cells. Th1 and Th2 effectors reactive to lymphocytic choriomeningitis virus (LCMV) were sorted according to the secretion of the effector cytokines interferon-g or interleukin-4/-10, respectively, by using cytokine secretion assay technology. Upon adoptive transfer into normal, nonlymphopenic recipient mice, Th1 and Th2 effectors efficiently gave rise to long-lived memory cells, demonstrating linear memory T-cell differentiation from naïve precursors via an intermediate effector state. Prior to viral infection, Th2 memory cells maintained high GATA-3 expression and produced Th2, but not Th1, cytokines upon reactivation. However, infection with Th1 cell-promoting LCMV reprogrammed Th2 cells to adopt a GATA-3^{+}T-bet^{+} 'Th2 + 1' hybrid state and coproduce Th2 and Th1 cytokines at the single-cell level. The Th2 + 1 hybrid phenotype was stably maintained *in vivo* for months, suggesting lineage-like properties. Th2 cell reprogramming *in vivo* and *in vitro* required TCR stimulation, concerted type I and type II interferon and interleukin-12 signals, and T-bet. Upon LCMV infection, virus-triggered T-bet induction in adoptively transferred Th2 cells was crucial to prevent viral persistence and fatal immunopathology. Thus, reprogramming of Th2 cells may facilitate the establishment of protective immune reactions. Stable coexpression of GATA-3 and T-bet provides a molecular concept for the long-term balanced coexistence of Th2 and Th1 lineage characteristics in single memory T cells.

609
Human Th17 plasticity

L. Cosmi,* F. Liotta,* L. Maggi,* V. Santarlasci,* M. Capone,* V. Querci,* R. De Palma,† E. Maggi,* S. Romagnani* & F. Annunziato*
**Department of Internal Medicine and DENOTHE Center, University of Florence, Florence, Italy,* †*Department of Clinical and Experimental Medicine, Second University of Naples, Naples, Italy*

Classically, CD4+ T-cells have been divided into Th1, which produce IFN-gamma, and Th2, which produce IL-4, IL-5 and IL-13. Recently, a third subset of T-cells which produce IL-17 has been described, the Th17 cells. We showed that, in addition to Th17, there is a number of T-cells that co-produce IL-17 and IFN-gamma (Th17/Th1). Th17 and Th17/Th1 cells express RORC and T-bet and they can be shifted to Th1 by IL-12. We also found that all IL-17-producing cells were included within the CD161+ fraction of circulating CD4+ T-cells. Moreover, UCB CD4+CD161+, but not CD4+CD161-, cells could be differentiated into IL-17-producing cells when activated in presence of IL-1beta plus IL-23. Finally, we identified CCR6+CD161+CD4+ T-cell clones showing the ability to coproduce IL-17A and IL-4 (Th17/Th2) and to induce IgE secretion *in vitro*. Very few Th17/Th2 cells were found among circulating CD4+ T cells from normal subjects, but their proportions were significantly increased in the circulation of chronic asthmatic patients. Th17/Th2 cells could not be derived from naïve UCB CD4+ T-cells under tested experimental conditions. However, cloning of CCR6+CD161+CD4+ T-cells in presence of IL-4 significantly increased the numbers of Th17/Th2 clones, suggesting that IL-4 may induce the shifting of memory Th17 cells into Th17/Th2 cells. The results of this study provide evidence of the plasticity of human Th17 cells even towards the Th2 phenotype.

282
Development and modulation of IL-17 immune responses

B. Stockinger, A. Potocnik & K. Hirota
Division of Molecular Immunology, MRC National Institute for Medical Research, London, UK

Interleukin 17 is a cytokine with central involvement in inflammatory immune responses. It is primarily produced by Th17 CD4 T cells, a subset of TCR$\gamma\delta$ T cells and some NKT cells. Over the past few years it has become clear that the production of IL-17 is regulated at multiple levels- both positively and negatively- to control its activity which when dysregulated can initiate pathogenic immunity and result in a variety of autoimmune syndromes. Cytokine production by Th17 effector cells is not a stable parameter, but instead appears to be modulated on several levels. As more states of CD4 T cell differentiation are uncovered, their flexibility is also beginning to be recognized. Components that control the plasticity of CD4 T cell populations include environmental influences at tissue sites, transcriptional circuitry and chromatin modifications. Understanding the rules that underlie adaptation and flexibility of T cell responses will be an essential part for future intervention strategies in human disease states.

Plenary Session 3: Found in Translation: Learning Immunology from Human Therapeutic Trials

882
Lessons learnt from the clinical use of anti CD52 antibodies

H. Waldmann
Sir William Dunn School of Pathology, University of Oxford, Oxford, UK

CAMPATH (Cambridge Pathology) 1 rat antibodies were first isolated in 1979 in the search for antibody targets that could exploit human complement, be able to selectively kill human lymphocytes whilst sparing hemopoietic stem cells. In the past 30 years studies with a series of IgM and IgG antibodies have given insights into:

1 Mechanisms of antibody mediated lysis, and the role of different antibody classes.
2 Insights into immunogenicity of antibodies and ways to minimise this.
3 Biological processes involved in marrow rejection and Graft versus Host disease.
4 Demonstration of an unsupected role of T-cells in disease cytopenias.
5 Evidence of long-term therapeutic benefit from short term lymphocyte ablation in diseases such as multiple sclerosis and in allogeneic renal transplantation.
6 Unexpected consequences of lymphocyte ablation that have encouraged underpinned screening programmes for individuals risking autoimmune disease following lymphocyte ablation.

This information has provided a basis for rationale intervention to favourably guide reconstitution of a lymphocyte purged immune system towards a better regulated one.

874
Reverse translation: lessons from the CD28 superagonist TGN1412

T. Hünig
Institute for Virology and Immunobiology, University of Würzburg, Würzburg, Germany

In 2006, a first-in-man study of the 'superagonistic' CD28-specific MAB TGN1412 resulted in an unexpected life-threatening cytokine release syndrome. Five years later, convincing explanations have been found for the failure of three sets of preclinical data (rodent, macaque and human PBMC) to warn of the impending disaster. A new *in vitro* assay for the study of T-cell activating biologicals will be presented which allows comprehensive biological and dose finding studies to soluble TGN1412. This system reveals initial cytokine release at about 2–5 % receptor occupancy, corresponding to a 20-fold lower mAb dose than was applied during the trial. Furthermore, it shows a remarkable similarity of TGN1412 and OKT3 with regard to cytokine profile, responding T-cell subset, and sensitivity of cytokine release to corticosteroid suppression.

852
New therapies in Alzheimer's disease – What have we learned?

R. Dodel
Neurology, Philipps University, Marburg, Germany

Alzheimer's disease (AD) is the most common neurodegenerative disorder leading to dementia and irreversible loss of neurons. The pathological hallmarks of AD are extracellular accumulation of amyloid-beta peptides (Aβ) as senile plaques and intracellular neurofibrillary tangles composed of tau proteins. Recently, active immunization in transgenic mice showed that immunization with Aβ peptides may help to inhibit the formation, promote clearance of Aß plaques and may also interfere with tau formation. In addition, passive immunization has also been shown to be effective on different outcome parameters in animal models of the disease. Shortly after those results, a clinical trial was initiated with an active immunization. Unfortunately, patients developed meningoencephalitis and thus the trial was stopped. Despite this setback, a large number of different approaches have been developed and some of them have already entered into clinical trials: six studies investigating active immunization approaches and nine studies evaluating passive immunization. Different epitopes, different kind of antibodies and different mechanisms were put forward to investigate the effect of immunization in AD. Recently, intravenous immunoglobulins have been tested in small pilot trials in this indication. The outcomes were favorable thus a large phase III trial was initiated in AD patients. As most of the studies will be finished in 2012 and 2013, the results will show whether immunization is able to prevent/halt/offset progression of AD.

In the presentation we will discuss the current issues in the different approaches and their assumptions and will review the current clinical trials in immunotherapy.

222
Trials and tribulations in targeting allergic pathways with biologics in asthma

S. Holgate
Infection, Inflammation and Immunity Division, Faculty of Medicine, University of Southampton, Southampton, UK

Traditional therapy of asthma disease has relied on reversal of symptoms with b2-adrenoceptor agonists and suppression of inflammation with corticosteroids. Beyond improving the pharmacology of known drugs, the only novel asthma therapies to emerge based on the allergic paradigm are leukotriene inhibitors (e.g. montelukast) and the non-anaphylactogenic anti-IgE, omalizumab, both directed to therapeutic targets identified over 40 years ago. However, there has been a myriad of new cellular and molecular targets discovered over the last 50 years that have formed the basis for new biologic therapies. A wide range of biologics targeting T cells, cytokines, chemokines adhesion molecules and inflammatory mediators all of which showed convincing efficacy in *in vitro* cell animal models and possibly some efficacy in acute allergen challenge in mild asthma have fallen short of expectations when trialled in human asthma. In moderate-severe asthma with the greatest unmet need, biologics against IgE, IL-4, IL-13, IL-5, IL-9 and TNF revealed only small subgroups in which efficacy has been shown or is suggestive. Treating asthma as a homogeneous disorder considered only by allergy and disease severity is the most likely reason for these disappointing trial results. The recent identification of serum periostin as a biomarker for the anti-IL-13 mAb lebrikizumab is a good example of things to come. In the future, to identify those patients most likely to respond to highly selective treatments such as biologics, it is essential asthma is defined by causative pathways so that appropriate diagnostic biomarkers can be developed.

Parallel Session 14: Autoimmune Disease: a Pathogen – Host Collaboration?

217
Viral infections and type 1 diabetes: good or bad?

M. G. von Herrath
Type 1 Diabetes Center – LIAI, La Jolla Institute for Allergy and Immunology, La Jolla, CA, USA

Type 1 diabetes (T1D) is a complex disease that is caused by an unfortunate combination of genetic predispositions and environmental influences. From studies in animal models we know that in particular viruses can enhance the disease course, especially if the pancreas and the islets are directly infected. In humans, infections with enteroviruses show an association with developing antibodies to islet antigens, thus supporting the concept that infections might provide a fertile field in those patients with a genetic predisposition to T1D. In our studies we have found high levels of MHC class I expression throughout human islets in patients with T1D, in many cases many years post diagnosis, which supports an involvement of viral infections (for example by pre-conditioning islets via interferon-mediated upregulation of MHC class I).

Animal models studies have taught us also, however, that viruses (and other infections, for example helminthes) can prevent diabetes. This occurs via several mechanisms, all of which involve the immune system's inherent property to return to baseline and maintain homeostasis: Apoptosis of aggressive lymphocytes via TNF, enhancement of regulatory T-cell (Treg) function involving TLRs and redirection of effector T cells to lymph nodes. These findings by others and our laboratory support the so-called 'hygiene hypothesis', which states that many infections are necessary for proper function and homeostasis of our immune system. Therapeutically it might be possible to transform deleterious into protective infections by antiviral vaccines and in this way possibly lower the incidence of type 1 diabetes.

645
Humoral immunity to microbial glycans in Guillain-Barré syndrome

H. Willison
Institute of Infection, Immunity and Inflammation, College of Biomedical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK

Guillain Barré syndrome (GBS) is the foremost cause of neuromuscular paralysis worldwide with a global incidence of ~$1.5/10^5$. The syndrome is a post-infectious autoimmune disease, often triggered by *Campylobacter jejuni* infection preceding neurological symptoms by ~2 weeks. Clinical-serological studies have identified serum anti-ganglioside antibodies in many GBS cases. Gangliosides are a family of ~50 structurally distinct sialic acid-containing glycosphingolipids highly enriched in the nervous system. Conclusive evidence indicates that these antibodies arise through molecular mimicry with sialic acid containing bacterial lipooligosaccharides (LOS), including those borne by particular strains of *C. jejuni*. Only a small proportion of *C. jejuni* infections trigger GBS, even when the ganglioside mimics are present in the LOS. The immune mechanisms responsible for antibody induction and a loss of host tolerance to LOS mimics are largely unknown and the subject of ongoing studies. More recently, antibodies to heteromeric glycolipid complexes have been identified and new combinatorial glycomic platforms have been designed for detecting these. Considerable emphasis is also placed on developing animal models of GBS based on either active immunisation with gangliosides or LOS, or passive immunisation with anti-ganglioside/anti-LOS antibodies, in combination with strategies to amplify the inflammatory milieu within the endoneurial compartment. Such models have been effectively used to develop and test novel therapeutic approaches. This presentation will highlight some of the recent progress being made in this field.

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Parasite modulation of autoimmune diseases

P. Zaccone,[*] O. T. Burton,[†] G. Schramm,[‡] H. Haas,[‡] M. J. Doenhoff,[§] D. W. Dunne[*] & A. Cooke[¶]
[*]Department of Pathology, University of Cambridge, Cambridge, UK, [†]Division of Immunology, Children's Hospital, Harvard Medical School, Boston, MA, USA, [‡]Research Center Borstel, Borstel, Germany, [§]The School of Biology, University of Nottingham, Nottingham, UK, [¶]Deaprtment of Pathology, University of Cambridge, Cambridge, UK

Although the majority of autoimmune diseases are under complex genetic control, environmental factors such as infections are playing an evident role in regulating autoimmunity. Studies in humans and in animal models demonstrated that infectious agents can both initiate/precipitate or prevent autoimmune disease. It has become evident that some infectious agents have evolved strategies to modulate host immune responses to facilitate survival of the pathogen while dampening down host pathology. These strategies often include modulation of cell subsets and cytokine/chemokine secretion by the host immune system. Parasitic worms like Schistosomes are master regulators of the host immune system, inducing functional and phenotypic changes in immune cells, generally inducing the expansion of Th2 responses. In the context of a Th1 or Th17 mediated autoimmune response the immunological signature induced by parasitic worms is shown to be useful to prevent of autoimmunity. The use of autoimmune disease animal models has highlighted the complex interplay between infectious agents and the host immune response. In animal models immunization with *Schistosoma mansoni* soluble antigen preparations can prevent experimental autoimmune encephalomyelitis (EAE), experimental colitis (EC) and protects non-obese diabetic (NOD) mice against the development of type 1 diabetes (T1D). These preparations generally induce the expansion of alternatively activated macrophages, tolerogenic DCs, iNKT cells, Th2 cells and Treg. Isolation and identification of the immunomodulatory molecules contained in such microbial extracts pave the way to the development of novel therapeutic strategies to prevent onset of T1D.

598

Helminthic infections, increased immune system tolerance and protection from IBD

J. V. Weinstock
Tufts University, Boston, MA, USA

Immunological diseases like IBD are infrequent in less developed countries possibly because helminthic infections provide protection by modulating host immunity. Therapeutic trials using helminths to control autoimmune disease show promise. The helminth Heligmosomoides polygyrus bakeri (Hp) prevents murine colitis. To study their mechanism of action, we used a Rag IBD model where animals were reconstituted with IL10-/- T cells to make them susceptible to IBD and with OVA antigen-responsive, transgenic OT2 T cells to allow study of gut antigenic responses. It was determine if altered DC function was a mechanism underlying IBD protection by *Hp*. Intestinal DC from *Hp*-infected Rag mice added to lamina propria mononuclear cells (LPMC) isolated from colitic animals blocked OVA IFNg and IL17 responses *in vitro* through direct contact with the inflammatory LPMC. Transfer of DC from *Hp*-infected mice into Rag mice reconstituted with IL10-/- T cells protected the animals from IBD, and LPMC from these mice lost OVA responsiveness. After DC transfer, OT2 T cells populated the intestines normally. However, the OT2 T cells were rendered antigen-nonresponsive through regulatory action of LPMC non-T cell elements. The DC did not function through altering Foxp3 T cell frequency. Thus, Hp modulates intestinal DC function, rendering them tolerogenic. This appears to be an important mechanism through which Hp suppresses colitis. Inappropriate T cell activation to luminal antigens may underlie the etiology of human IBD, and IFNg and IL17 are colitogenic. Thus, the capacity of these DC to block a gut antigen-specific IFNg/IL17 T cell response also is significant.

Parallel Session 15: Recent Advances in T Cell Signalling

30
Initiation and propagation of the signalling wave within TCR nanoclusters

A. Borroto, R. Blanco & B. Alarcon
Centro de Biologia Molecular Severo Ochoa, Madrid, Spain

Ligand binding to the TCR induces a conformational change a hallmark of which is the exposure of the proline-rich sequence (PRS) in CD3e that permits Nck binding through its SH3.1 domain. We show that mutation of the PRS prevents mice from mounting an adaptive immune response *in vivo*; manifested by hypersensitivity to viral infection and incapacity to acquire immunity to a tumor after vaccination. Moreover, the rearrangement of the actin cytoskeleton and CD3 phosphorylation induced by the TCR are significantly compromised. Furthermore, a high-affinity peptide inhibitor of the SH3.1 domain impairs assembly of the TCR signalosome. These results suggest that Nck recruitment is a fundamental early step in TCR signaling, this event representing a target to modulate the immune response.

Although Nck recruitment is a consequence of the conformational change in the TCR it might not be the only one. To understand the importance of the conformational change in the TCR on T cell differentiation and T cell activation, we have generated a knockin mutant bearing a C80G mutation in the CxxC motif of the ectodomain of CD3e. This mutation abrogates transmission of the conformational change from the TCRa/b heterodimer to the CD3 cytoplasmic tails. Thymocytes from mice homozygote for the C80G mutation are completely arrested at the double negative DN3 stage ($CD4^-CD8^-CD25^+CD44^-$), indicating that the mutation abrogates pre-TCR activity. Since the pre-TCR is supposed to signal in the absence of interacting ligands, we conclude that the pre-TCR must be in a constitutive active conformation in wild type pre-T cells.

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Chemical-genetic approach to turning T cells on or off with selective tyrosine kinase inhibitors

A. Weiss
Medicine, HHMI/UCSF, San Francisco, CA, USA

T cell antigen receptor (TCR) signal transduction is initiated by the sequential and regulated action of the Lck and ZAP-70 cytoplasmic tyrosine kinases. Because of the importance of ZAP-70 in normal and pathological conditions, ZAP-70 would seem to be an attractive therapeutic target. By mutating the gatekeeper residue and using bulky PP1 analogs have developed a genetically-selective system for ZAP-70 inhibition. Results from this system support the notion that ZAP-70 catalytic function is critically important in most, but perhaps not all, mature peripheral T cell functions. T regulatory cell function is resistant to the inhibition of ZAP-70 catalytic function and, instead, depends upon the scaffolding function of ZAP-70. These results validate ZAP-70 as an attractive therapeutic target.

The Src family kinase Lck is also critical for the initiation of TCR signaling function and of ZAP-70 function. In T cells, Src kinases are regulated, in part, by the opposing actions of the receptor tyrosine phosphatase CD45 and the cytoplasmic tyrosine kinase Csk. We have applied the genetically-selective inhibitor system to probe the importance of Csk function. These studies suggest a very dynamic and highly adaptable mechanism of controlling Src kinase activity in the basal state. Inhibition of Csk function can lead to activation of TCR signaling in the absence of TCR ligands.

Thus, we demonstrate the utility of probing TCR signaling function negatively and positively with genetically-controlled systems with tyrosine kinase inhibitors.

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Signalling by Ras and PI3Ks in early T cell development

M. Turner
Lymphocyte Signalling and Development, Babraham Institute, Cambridge, UK

Thymocytes are tested for productive rearrangement of the *tcrb* locus by expression of a preTCR in a process termed beta-selection which also requires signalling by Notch1. Recently, we fund a role for the chemokine receptor CXCR4 and its ligand CXCL12/SDF1 in beta-selection. We show that PI3K signaling from the preTCR is mediated in part by the p110delta phosphatidylinositol 3-kinase (PI3K). By contrast, CXCR4 signalling is mediated by p110gamma PI3K. Mice with a mutant allele of p110gamma unable to bind active Ras revealed that CXCR4-mediated PI3K activation is Ras-dependent. The Ras-p110gamma interaction was necessary for efficient beta-selection-promoted proliferation but was dispensable for the survival or differentiation of thymocytes. Inclusion of recombinant CXCL12 allows for Notch1-dependent differentiation of DN3a or DN3b thymocytes in the absence of supporting stromal cells. Inclusion of a GSK3 inhibitor allowed PI3K-dependent accessory cell free proliferation and differentiation of DN3 cells mimicking aspects of beta-selection *in vitro*.

Parallel Session 16: Stroma Cells: Masters of the Immune System

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Immune and metabolic functions for the stromal cell expressing fibroblast activation protein (FAP)

M. Kraman,* E. Roberts,* J. Arnold,* J. Jones,* C. Feig,† D. Tuveson† & D. Fearon*
**University of Cambridge, Cambridge, UK,* †*CRUK Cambridge Research Institute, Li Ka Shing Centre, Cambridge, UK*

A challenge for tumor immunology is to understand local immune suppression within the tumor. Twenty years ago Old identified a stromal cell that was present in essentially all human adencarcinomas, which was identified by its expression of a membrane protein, termed, 'Fibroblast activation protein-alpha' (FAP). This stromal cell was also found in a variety of lesions that can be characterized as non-infected, chronic inflammation, and in the uterus and placenta. A unifying explanation for the presence of the FAP+ stromal cell in tumors and chronic 'wounds' would be that it promotes healing by suppressing immunological tissue damage. To determine whether the FAP+ stromal cell accounted for tumoral immune suppression, we generated a genetically modified mouse in which the human diphtheria toxin is expressed in FAP-expressing cells to permit conditional depletion by diphtheria toxin. Depleting FAP+ cells in mice bearing established, ectopic, spontaneously immunogenic tumors led to immune control of tumor growth. This outcome has been recently replicated in a mouse model of spontaneous pancreatic adenocarcinoma. These findings suggest that depletion of FAP+ cells may enhance immunotherapy of human cancer, if FAP+ stromal cells do not have essential functions in normal tissues. We have discovered with mouse in which luciferase is expressed in FAP+ stromal cells that these cells reside in many normal tissues. In some of these they have important homeostatic functions. Therefore, FAP identifies not only a tumoral stromal cell that mediates tumoral immune suppression, but also a cellular lineage with essential normal functions.

653
The stromal niche for CD4 memory

P. J. Lane, F. M. McConnell, F. M. Gaspal, G. Anderson & D. Withers
MRC Centre for Immune Regulation, IBR, Birmingham, UK

Mammalian phylogeny shows that the capacity to mount CD4 dependent memory antibody responses co-evolved with lymph nodes in placental mammals. In the embryo of all placental mammals, lymphoid tissue inducer cells (LTi) co-ordinate lymph node development through expression of mammalian restricted ligands for the lymphotoxin beta receptor (LTbR), expressed by lymph node stromal cells. Our studies indicate that LTi persist in adult lymph nodes, and express in addition to LTbR-ligands, high levels of the related tumor necrosis factor (TNF) member ligands for OX40 and in mouse but not man CD30. Our studies indicate that LTi are critical for the persistence of CD4 memory, and that specifically CD4 memory cells encounter LTi and receive OX40 and CD30 survival signals as they recirculate through lymph nodes. In this presentation we will define the stromal niche that fosters the interactions between LTi and CD4 memory T cells, and compare and contrast the recquirement for OX40 and CD30 signals in the survival of CD4 memory and effector cells, as the latter also depend on these signals. Specifically our studies provide a cellular mechanism for the independent regulation of the effector and memory CD4 pools.

44
Lymph node fibroblasts as key players in adaptive immunity

S. A. Luther
Department of Biochemistry, University of Lausanne, Epalinges, Switzerland

Secondary lymphoid organs such as the spleen and lymph nodes are the only sites where primary immune responses against pathogens are efficiently initiated. These organs are compartmentalized with T and B lymphocytes residing in distinct zones. In the last few years it has become clear that this compartmentalization is achieved by specialized resident fibroblast subsets which constitutively produce chemotactic factors. While B zone stromal cells (such as follicular dendritic cells) have been characterized extensively and attributed many important functions, their T zone counterpart, called fibroblastic reticular cells (FRC), has been poorly characterized. For a long time FRCs were proposed to produce extracellular matrix and to represent simply the structural backbone of the tissue. Over the last few years FRC have been shown to play several active roles in adaptive immunity. I plan to discuss our recent observations on the role of FRC during T cell responses.

626
In vivo imaging of HEVs and lymphatic vessels in lymph nodes and ectopic (tertiary) lymphoid tissues

K. L. Bentley, L. A. Truman, N. Alonso-Gonzalez & N. H. Ruddle
Yale University, New Haven, CT, USA

Dynamic changes occur in the lymph node vasculature after immunization that can affect antigen priming. After immunization, there is a temporary defect in lymph-flow through the afferent lymphatic vessels and high endothelial venules (HEVs) revert to an immature phenotype Later, intense lymphangiogenesis occurs that is regulated in part by B cells and HEVs recover. In order to visualize these changes in real time, we have made transgenic mice with red-fluorescent lymphatic vessels and green fluorescent HEVs that are suitable for *in vivo* imaging. The lymph nodes of ProxTom/Hec6stGFP reporter mice were successfully imaged using 2-photon laser-scanning microscopy allowing simultaneous visualization of lymphatic vessels and HEVs. Migration of lymphocytes from the HEVs to the parenchyma of lymph nodes was also imaged. Chronic inflammation that occurs in autoimmunity, cancer, and microbial diseases can assume the appearance of lymph nodes. These tertiary lymphoid organs (TLOs) contain HEVs, lymphatic vessels, lymphoid chemokines, T and B cell compartmentalization, antigen presenting cells, and germinal centers. We are studying lymphatic vessels and HEVs in several TLO mouse models of human diseases, including Sjögren's syndrome and multiple sclerosis. The ProxTom/Hec6stGFP reporter mice will be useful tools for imaging the vasculature of TLOs found in target organs in chronic inflammation.

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Parallel Session 17: CD8 T Cells in Anti-Viral Immunity and Therapy

878
Success and failure of virus-specific CD8+ T cell responses in hepatitis C virus infection

R. Thimme
Department of Medicine II, University Hospital Freiburg, Freiburg, Germany

Hepatitis C virus (HCV) infection is only cleared in a minority of infected individuals, the majority of patients develop chronic infection. Chronic HCV infection potentially leads to liver fibrosis, cirrhosis and finally hepatocellular carcinoma. The host immune response is an important determinant in the outcome of HCV infection. Innate as well as adaptive cellular and humoral immune responses mediate important antiviral actions; however, virus-specific T cell responses appear to be most critical. Indeed, strong and multispecific CD4+ as well as CD8+ T cell responses are required for viral clearance. In most individuals, however, the HCV-specific immune response fails to clear the virus. Several mechanisms underlying this HCV specific T cell failure have been identified. These include viral factors such as viral escape mutations and immunological factors such as the expression of inhibitory receptors, which lead to CD8+ T cell dysfunction. An in-depth understanding of the determinants of success or failure of the HCV-specific T cell response is critical for the development of prophylactic as well as therapeutic vaccination regimes against HCV.

856
How HBV exploits the intrahepatic immune environment

M. Maini
UCL, London, UK

T cells are critical for control of the non-cytopathic hepatitis B virus but are depleted and functionally impaired in patients with persistent infection. I will describe mechanisms leading to the deletion and exhaustion of antiviral T cell responses, with an emphasis on the contribution of the liver environment. NK cells are enriched and activated in the liver but are also functionally polarised, with an impairment in their non-cytolytic antiviral function. Current antiviral therapy is unable to adequately re-programme anti-HBV immune responses; novel approaches to selective immunological re-programming and the risks of breaking hepatic tolerance will be discussed.

789
HIV protective immunity: illustrations from HIV-2 infection

S. Rowland-Jones
Weatherall Institute of Molecular Medicine, Oxford University, Oxford, UK

Despite between 30% and 60% sequence homology between HIV-1 and HIV-2, HIV-2 infection is largely confined to West Africa and countries with cultural or economic links to Portugal and has not led to a world-wide epidemic. Indeed recent data suggest that the prevalence of HIV-2 infection is declining across West Africa. HIV-2 infection is characterized by an attenuated disease course that distinguishes it from HIV-1. However, this is not due to slow disease progression in all subjects. Longitudinal studies in the Caio community HIV-2 cohort in Guinea Bissau have shown that close to 40% HIV-2-infected subjects behave as Long-term non-progressors (LTNPs), maintaining an undetectable viral load for a decade or more, whereas 15–20% progress to disease in a manner clinically indistinguishable from people infected with HIV-1. Thus HIV-2 presents the intriguing picture of a retroviral infection that is compatible with a normal lifespan yet can cause AIDS and death in some people.

The results of studies in the Gambia and Guinea-Bissau will be presented that demonstrate that host genetic and immunological features correlate with viral control, particularly HLA, KIR and TRIM polymorphisms, together with cellular virus-specific responses (but not the very potent neutralising antibody response). These findings may provide important information for vaccination strategies for HIV-1.

Parallel Session 18: Evolutionary Immunology

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The origin of the adaptive immune system of vertebrates: a bird's eye view

J. Kaufman[*,†]
[*]*Pathology, University of Cambridge, Cambridge, UK,* [†]*Veterinary Medicine, University of Cambridge, Cambridge, UK*

The immune response is enormously complex, due to evolution involving step-by-step acquisition of protective mechanisms adapted from different molecular and cellular processes, each step in response to particular challenges and overall taking millions of years. Reconstructing events to understand fundamental processes and principles underlying evolution of immune responses is difficult.

One part amenable to analysis is adaptive immunity, clearly a 'system' with a unique origin. Work by many people revealed that key genes and processes in mammals were already in place at the emergence of jawed vertebrates. Analysis of jawless fish at the base of vertebrates suggests that the whole system is older yet.

In contrast to mammals, the chicken MHC has strong genetic associations with resistance to infectious pathogens and responses to vaccines. We found that chickens have single dominantly-expressed class I and class II molecules, whose properties can determine the immune response. The basis for the dominantly-expressed class I gene is co-evolution with highly polymorphic TAP and tapasin genes located nearby. Unexpectedly, NK receptor (NKR) genes are also present in the chicken MHC.

These salient features are found in many non-mammalian vertebrates, suggesting the ancestral organisation was like chickens, with the mammalian MHC arising by a messy inversion. The presence of NKR genes suggests that receptors were also present in the primordial MHC in order to co-evolve with their ligands. Many other disparate data are explained by this view of the primordial MHC as the birthplace of the adaptive immune system, which has been falling apart ever since.

351
Evolution of a lymphocyte-based adaptive immune system

M. D. Cooper, S. Das, P. Guo, M. Hirano, B. Herrin, J. Li, H. Nakahara, N. McCurley, A. Sadlonova & C. Yu
Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA, USA

The alternative adaptive immune systems found in jawed and jawless vertebrates have remarkable similarities, but they differ radically in composition of their antigen receptors. Leucine-rich-repeat (LRR)-based variable lymphocyte (VLR) receptors are used for antigen recognition by jawless vertebrates, whereas Ig-based TCR and BCR are used for the same purpose by jawed vertebrates. Lampreys, the best studied jawless vertebrates, have diverse repertoires of VLRA, VLRB and VLRC types of anticipatory receptors that are expressed by separate lymphocyte lineages. The three germline VLR genes are incomplete, typically coding only portions of the amino- and carboxy-terminal LRR regions plus the invariant stalk region. However, hundreds of different *lrr* sequences that flank the incomplete *VLR* genes can serve as template donors to complete a *VLRA, VLRB* or *VLRC* gene during lymphocyte differentiation. Monoallelic VLR assembly is associated with expression of AID-APOBEC orthologs, cytidine deaminase 1 (CDA1) or CDA2. $VLRA^+$ lymphocytes respond to antigenic stimulation, but do not secrete their receptors. $VLRB^+$ lymphocytes respond to cognate antigens with proliferation and differentiation into plasma cells, which secrete multimeric VLRB antibodies with either protein or carbohydrate antigen specificity. Activated $VLRA^+$ cells upregulate their expression of interleukin-17, the receptor for which is expressed by $VLRB^+$ cells. Conversely, activated $VLRB^+$ cells upregulate IL-8 expression and $VLRA^+$ cells express the IL-8R. These findings suggest that $VLRA^+$ and $VLRB^+$ lymphocytes are functionally interactive. Currently available information suggests that the lamprey $VLRA^+$ and $VLRC^+$ lymphocytes belong to separate T-like lineages, much like our $\gamma\delta$ and $\alpha\beta$ T cell lineages.

249
The adaptation of chronic viral infections to human genetic diversity

O. Pybus & P. Markov
Department of Zoolgy, University of Oxford, Oxford, UK

The adaptation of viruses that establish chronic infections to the immune responses of their hosts is well documented. Notably, previous studies have observed the predictable evolution of HIV-1 escape mutations in epitopes targeted by effective host CTL responses. However, the long-term evolutionary fate of such escape mutations is unknown. Here I introduce a new framework that combines evolutionary and immunological data and show how it reveals the historical adaptation of hepatitis C virus genomes to different human populations. The results illustrate that an evolutionary perspective can improve our understanding of HCV disease progression and treatment response.

635
Immunoheterogeneity and immunosenescence in the wild: an evolutionary ecologist's perspective

D. Nussey
Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, UK

There is marked variation in disease susceptibility and immune response profiles both among individuals and within individuals across their lifetimes, and much of this variation appears to have a genetic basis. Understanding both the origins and the health and fitness consequences of such heterogeneity is a central concern in both immunology and evolutionary ecology. Evolutionary explanations hinge on the idea of fitness costs of immunity. While a robust immune response is considered crucial to survival in parasite-filled natural environments, this response may draw limited resources away from other vital fitness functions (e.g. growth or reproduction) or actually directly cause damage (e.g. immunopathology). To understand how natural selection actually shapes among-individual and age-related variation in immunity, it is crucial to test such predictions in populations experiencing evolutionarily realistic conditions. I will discuss recent work examining variation in circulating antibody levels and T cell subsets in a wild population of Soay sheep on the St Kilda archipelago, which has been the subject of individual-based study for several decades. Our results illustrate how complex and counter-balancing relationships between immune measures and fitness could maintain genetic variation in immunity. We also find similar patterns of age-related variation in immunity in nature to those seen in lab mice and humans, opening the way for research in this and similar systems to address how early-life decisions and experiences might impact on immunosenescence, and how natural selection might shape immune variation across the entire lifespan of individuals.

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How do hosts balance resistance, tolerance, repair and resilience to infections?

D. Schneider

Microbiology and Immunology, Stanford University, Stanford, CA, USA

We are trying to understand how a host balances its ability to clear a pathogen (resistance) with its ability to endure an infection (tolerance). We've been working with an ecological definition of the word 'tolerance' where it is defined as the dose response of elicitor with respect to the impact on health. We've found that hosts can vary in their resistance and tolerance and are trying to define molecular mechanisms for both traits. The problem with tolerance is that it can only be measured for a population and not individuals. We recently developed an approach to plot health-by-microbes in phase space that lets us look at the relationship between health and microbe numbers in individuals. This approach lets us focus on repair and resilience to infections and gives us a new method of analyzing the multivariate data that we can gather in infected individuals, from flies to mice to people.

Session 19: Lost in Translation? How we Ensure Useful Transit of Diagnostic Tests from Research Studies to Clinical Practice

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'Ten things I hate about tests' (why they don't always mean what you think they do)

W. Egner
Clinical Immunology and Allergy, Sheffield Teaching Hospitals UKNEQAS, Sheffield, UK

Management of patients increasingly relies on diagnostic triaging or screening to allow access to patient care pathways in the most cost-effective and clinically-effective manner. Medical staff, research scientists and patients generally have a touching faith in tests which is often not matched by their performance in real life. It is not uncommon for enthusiastic adoption to fail to deliver the performance suggested in initial experiments and surveys. There is an extensive literature and experience with these issues in diagnostic laboratories and EQA providers which is often not utilised effectively in constructing pathways and guidelines. It is rare that the end user is aware of most of these issues. Often guidelines are written which expect performance that cannot be delivered consistently or at all. This presentation will explore the reasons for this with examples and will explore potential solutions.

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The evolution of the disease-specific immune response in (pre)-arthritis: can we identify a Masterswitch determining the onset of rheumatoid arthritis?

R. Toes
Rheumatology, Leiden University Medical Centre, Leiden, The Netherlands

Anti-citrullinated protein antibodies (ACPA) are a very distinctive feature of rheumatoid arthritis (RA) patients. The presence of these antibodies is highly predictive for both the development of RA and the extent of associated joint destruction. Recent evidence indicates that well-known genetic risk factors for RA: the HLA-DRB1 shared epitope (SE) alleles and the PTPN22 T-allele are predominantly associated with anti-CCP positive RA. These reports, together with the finding that ACPA can exacerbate arthritis in mice, suggest that anti-peptidylcitrulline immunity plays an important role in the pathogenesis of the disease.

Studies investigating at which point in time ACPA first appear, have revealed that these antibodies can often be detected several years before disease onset. The mere presence of ACPA therefore does not appear to be sufficient to precipitate disease. An explanation for this observation could be that the anti-citrullinated protein immune response first needs to mature more fully, in the course of which ACPA could acquire distinct characteristics which are instrumental in mediating tissue damage.

Taking into consideration that ACPA can be detected before the clinical diagnosis of RA, and that the presence of ACPA is strongly associated with disease progression, we hypothesized that epitope spreading, avidity maturation and/or isotype usage of the ACPA response may play a role in the evolution of the disease.

Our data reveal that broadening of isotype-usage and Epitope spreading with recognition of more citrullinated antigens occurs before onset of RA and that immunological differences in these ACPA are associated with the future disease course.

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The role of anti-citrullinated peptide antibodies in the diagnosis and management of rheumatoid arthritis

A. G. Wilson
Infection and Immunity, University of Sheffield, Sheffield, UK

Rheumatoid arthritis (RA) is the commonest autoimmune inflammatory joint disease with a prevalence of 1%. Although significant advances in treatment have occurred over the past 15 years significant challenges remain including earlier diagnosis, improved patient stratification and therapeutic targeting. The discovery of anti-citrullinated protein antibodies (ACPA) has given important insights into the pathogenesis of RA; it is associated with smoking and the DRB1 shared epitope revelaing an important gene-environment interaction in the RA pathogenesis. In addition many of the recently identify RA susceptibility loci are associated with ACPA +ve RA only. Additional evidence that ACPA status defines a distinct form of RA is the contrasting synovial infiltrate; ACPA+ve synovium has greater CD3, CD8, CD45 RA and CXCL12 expression and lymphocytic infiltrate but reduced fibrosis and synovial lining thickness compared with ACPA-ve synovium. As a diagnostic aid ACPA is less sensitive than rheumatoid factor (RF) (65% v 70% respectively) but more specific (99% versus 94%), it is particularly useful in predicting progression to RA in cases of undifferentiated inflammatory arthritis. Joint damage is higher in ACPA+ve RA particularly if RF is also present. Biological therapies are highly efficacious but there use is limited by cost and up to 30% of patients fail to have a satisfactory response to anti-TNF therapies. Recent studies have suggest that ACPA+ve RA responds better to anti-CD20 therapy compared with ACPA-ve disease but the reverse is true for anti-TNF agents. Thus ACPA status is a useful diagnostic, prognostic and therapeutic biomarker in RA.

Parallel Session 20: The Immunology of Rheumatology

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The immunology of Ankylosing Spondylitis

P. Bowness
NDORMS, Oxford University, Oxford, UK

Ankylosing Spondylitis is an immune-mediated rheumatic disease with a major genetic contribution, and a likely ubiquitous environmental trigger. The role of the Human Leukocyte Antigen HLA-B27 has been known for more than 30 years. Recent GWAS studies have shown that ERAP polymorphisms are important in B27+ cases (implicating antigen presentation), and have also implicated cytokine pathways including the Th17 pathway. The talk will discuss the immune cells, cytokines and genes implicate in AS, before moving on to the role of HLA-B27.

HLA-B27 may cause AS because it has an abnormal cell biology. Thus B27 can form homodimers ($B27_2$) on the cell surface. Cell surface $B27_2$ may have a pathogenic role in SpA by binding Natural Killer family receptors, including KIRs (killer Ig-like receptors) and/or LILRs (leukocyte Ig-like receptors). We have shown that, in SpA, an increased number of peripheral blood NK and CD4 T cells express the KIR3DL2 receptor compared to healthy controls and RA patients. These cells are enriched for IL17 production. Apoptosis of $KIR3DL2^+$ NK and T cells *ex vivo* can be inhibited by co-culture with $B27_2$ -expressing cells.

Understanding the immune pathogenesis of AS will give great opportunities for more effective targeted therapy. In turn the efficacy (or otherwise) of biological therapies including anti-TNF, anti-B cell and anti-Th17 in treatment trials gives invaluable information about the roles of these pathways in disease.

572
SLE and biologic therapies

B. Diamond
Autoimmune and Musculoskeletal Diseases, The Feinstein Institute for Medical Research, Manhasset, NY, USA

Systemic lupus (SLE) is an autoimmune disease characterized by the production of autoantibodies. Mouse models demonstrate that primary defects in B cells, T cells, or dendritic cells can all lead to a lupus-like syndrome in mice, suggesting here may be multiple therapeutic targets in SLE. The entry of biologic therapies into the treatment of SLE has, however, been somewhat disappointing. Some problems in translating from mouse to human may relate to

1 an incomplete consideration of the biology,
2 an inadequate approach to patient phenotyping and
3 to imprecise measures of clinical outcome.

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Animal models of rheumatoid arthritis

C. A. Notley
Centre for Rheumatology, University College London, London, UK

The use of animal models to study the initiation, progression or resolution of human disease is a widely accepted tool in many fields, including rheumatology. Indeed, these models have been pivotal in the development of biologic therapy to treat rheumatoid arthritis (RA). There are a number of animal models mimicking different aspects of RA pathogenesis, these include inducible models such as collagen-induced arthritis (CIA) and antigen-induced arthritis (AIA), and spontaneous models such as the SKG and the KRN models.

CIA, which is a chronic, systemic, polyarthritis involving the activation of neutrophils, T and B cells, is the most widely used model for testing potential therapeutic agents, such as anti-TNF-alpha and anti-CD3 therapy. However, acute inflammatory models (e.g. AIA) can be easily manipulated allowing for studies such as those testing the antigen specific suppression of disease by antigen targeted CD4+Foxp3+ Tregs.

Spontaneous mouse models have been primarily studied to dissect the pathogenesis of inflammatory arthritis, and the importance of cell subsets such as Th1 and Th17 cells in the initiation and progression of disease. It is the ability to translate findings from these diverse murine models to understand disease and develop novel therapies for patients that is key to their successful exploitation.